PRIMARY CORONAL CARIES PREVENTION WITH SILVER DIAMINE FLUORIDE – INVESTIGATIONS INTO EFFICACY AND MODE OF ACTION

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DEDICATION

To my father Ramezan, my mother Touran,

To my sisters Yassaman and Parissai.

To my nephew Parsa.

To my long-standing friend; Sasan Hashemi.

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Parand Sorkhdini

PRIMARY CORONAL CARIES PREVENTION WITH SILVER DIAMINE FLUORIDE – INVESTIGATIONS INTO EFFICACY AND MODE OF ACTION

Dental caries continues to be one of the most prevalent preventable diseases worldwide. Silver diamine fluoride (SDF) is a topical solution comprised of silver, ammonia and fluoride. It is a safe, effective, efficient, noninvasive and cost-effective method in caries management. However, there is little clinical evidence supporting the use of SDF (or SDF followed by application of potassium iodide[KI] to mitigate staining) as anti-caries agents on sound enamel and early enamel carious lesions. In this dissertation, I studied the mechanism behind SDF's ability to prevent coronal caries which has not been studied yet. In the first and second aims, I investigated the effectiveness of SDF, SDF+KI, fluoride (potassium fluoride [KF]) and silver (silver nitrate [$AgNO_3$]) controls to SDF and deionized water (DIW) in preventing enamel demineralization and enhancing remineralization using chemical, biofilm and pH-cycling models. In both chemical demineralization and pH-cycling models there were no statistically significant differences between SDF and SDF+KI in preventing coronal caries. In the biofilm model, however, SDF+KI was significantly less effective in preventing demineralization than SDF. In the third aim, I investigated the efficacy of SDF, SDF+KI, KF, $AgNO_3$, and DIW on the remineralization of active subclinical enamel carious lesions. Here, SDF+KI was significantly more effective in promoting remineralization than SDF.

I calculated changes in color, and the results show applying KI after SDF significantly reduced the dark staining caused by SDF. In conclusion: SDF and SDF+KI

appear to be effective options in preventing and in the treatment of primary coronal caries. Further clinical research is required to confirm the present findings.

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LIST OF ABBREVIATIONS

SDF: Silver Diamine Fluoride
KI: Potassium Iodide
$AgNO_3$: Silver Nitrate
KF: Potassium Fluoride
DIW: Deionized Water
SMH: Surface Microhardness
TMR: Transverse Microradiography
IRB: Institutional Review Board
USA: United States of America

CHAPTER 1: GENERAL INTRODUCTION

There are several risk factors for dental caries including biological, physical, environmental, behavioral and lifestyle-related factors, such as high levels of cariogenic bacteria, dry mouth, insufficient fluoride exposure, limited access to dental care and poor oral hygiene. Dental caries is caused by a complicated interaction between acid-producing bacteria in the biofilm that ferment dietary carbohydrates and several oral environmental factors over time. These shift the equilibrium from remineralization (mineral gain) towards demineralization (mineral loss) (Selwitz, Ismail et al. 2007, Vinh N 2017).

Over the last 20 years, a high prevalence of dental caries, especially among children, and the increasing cost of healthcare pose a significant public health problem all over the world. This is exacerbated because the current treatment approaches are limited in their effectiveness, and health care barriers still exist in delivering effective caries control and prevention (Crystal and Niederman 2016). Host susceptibility, oral pathogens, and dietary carbohydrates are the most important factors responsible for dental caries development (Moynihan and Petersen 2004). Among oral pathogens, the significance of *Streptococcus mutans (S. mutans)* as one of the prime etiological agents in the development of dental caries has been studied widely. Several clinical cross-sectional studies have demonstrated that there is a correlation between counts of *S. mutans* and *lactobacilli* in saliva or plaque and a high level of caries incidence (Featherstone 1999).

The current paradigm for management of dental caries is based on preventing demineralization and encouraging remineralization of the lesion at the earliest phase. To date, besides dental restoration, no other treatment option for dental caries has displayed

significant efficacy (Horst, Ellenikiotis et al. 2016). Research is still ongoing to manage the dental caries process over time for individual patients, with a minimally invasive, tissue-preserving approach (Selwitz, Ismail et al. 2007). The efficacy of various fluoride caries-preventive agents, including sodium fluoride (NaF) varnish, acidulated phosphate fluoride (APF) gel, 2% neutral fluoride gel, stannous fluoride gel (SnF_2; which is no longer used routinely due to staining), and amine fluoride preparations (AmF) have been evaluated (Savas, Kucukyilmaz et al. 2015). It has been shown that multiple applications of fluoride enhanced not only the hardness of the tooth structure but also had preventive effects against initial dental caries (Byeon, Lee et al. 2016). Nevertheless, these agents have not shown a satisfactory anti-caries efficacy, and efforts to create more effective anti-caries agents are still ongoing and desperately needed (Savas, Kucukyilmaz et al. 2015).

Silver diamine fluoride (SDF)

The silver diamine fluoride ($Ag(NH_3)_2F$) solution is a colorless aqueous solution which contains both silver fluoride (AgF) and ammonia (NH_3). Due to their penetrative abilities, silver and fluoride ions diffuse ~25 μm into sound enamel and 50-200 μm into sound dentin (Horst, Ellenikiotis et al. 2016). The equation for a chemical reaction of SDF has been suggested to be:

$$Ag(NH_3)_2F(aq) \rightarrow Ag(s) + 2NH_3(g) + F^-(aq)$$

The free fluoride ions promote remineralization of dentin and enamel lesions; however, the excess antimicrobial silver ions can be precipitated as Ag_2S. These silver precipitates leave a black staining on the teeth that present cosmetic issues for patients. One possible solution to minimize staining is to apply a potassium iodide (KI) solution

immediately after SDF application to bind free silver ions from SDF. The equation for a chemical reaction of SDF applied with KI has been suggested to be:

$$Ag(NH_3)_2F(aq)+KI(aq)\rightarrow AgI(s)+2NH_3(g)+F^-(aq)$$

KI reacts with free silver ions and forms a yellow precipitate of AgI, which can be easily rinsed away, and prevents the black staining caused by SDF. Therefore, when KI solution is applied after SDF application, the extra silver ions are removed (Vinh N 2017). In this study, the central hypothesis was that applying KI immediately after SDF solution will reduce the staining development associated with SDF. In addition, I wanted to investigate whether adding KI to SDF can reduce the anti-caries efficacy of SDF.

In-vitro studies have shown that SDF prevents the formation of mono-species biofilms of *S. mutans* and *Actinomyces naeslundii* and that it also helps to prevent dentin demineralization (Mei, Li et al. 2013). The antibacterial properties of Ag ions and fluoride to treat dental caries have been shown, and fluoride encourages remineralization with ammonia helping to stabilize AgF in the solution through complexation in a reversible reaction (Liu, Lo et al. 2012). It has been suggested that SDF has effective antibacterial properties and is capable of decreasing enamel surface mineral loss and increasing enamel surface microhardness (Savas, Kucukyilmaz et al. 2015). SDF or AgF can increase the acid resistance of enamel by reducing the enamel solubility in acids or by increasing fluoride combination in the enamel (Delbem, Bergamaschi et al. 2006).

The overall objective was to develop a fundamental understanding of the effects of SDF and its individual components, Ag and fluoride ions, on enamel de- and remineralization. Previous studies on the anti-caries ability of SDF have focused primarily on dentin caries. Thus, this research was innovative for studying enamel caries.

The long-term goal in this proposed study was to determine the efficacy of SDF and its components, Ag and fluoride, in isolation. I provided a clear and precise understanding about the role of fluoride and silver ions in the arrestment of early enamel caries lesions and the prevention of primary enamel caries.

The main goal of this investigation was to investigate the efficacy of the application of SDF on the inhibition of enamel demineralization of sound enamel, as well as promotion of remineralization and prevention of secondary demineralization of early enamel caries lesions. I included appropriate controls to SDF as I studied the effects of Ag (as $AgNO_3$) and fluoride (as KF) in isolation. Furthermore, I studied the effect of a post-SDF application of potassium iodide (KI), which is commonly used to lessen the expected staining caused by Ag. Deionized water (DIW) served as a negative control. The long-term goal in this proposed study was to design a new standard protocol to be used as a basis for SDF application for the arrestment of early enamel caries lesions and the prevention of primary enamel caries.

The hypotheses of my first study were that a) SDF is an effective anti-caries agent in the inhibition of enamel demineralization, b) KI application immediately after SDF treatment can significantly reduce staining caused by SDF alone while not affecting SDF anti-caries efficacy, and c) SDF may be comparatively more effective in inhibiting demineralization in a biofilm model than in a chemical model.

For the second project, I hypothesized that a) SDF is still effective in enamel caries prevention with twice-daily fluoride application, and b) applying KI after SDF application can mitigate dark staining and at the same time does not negatively affect the anti-caries ability of SDF.

For the third project, I hypothesized that a) SDF is an effective agent in promoting remineralization, b) applying KI after SDF application can lessen dark staining while not adversely affecting the remineralization promotion efficacy of SDF and, c) mucin in AS will enhance the ability of SDF to promote remineralization of early caries lesions.

To assess theses hypotheses, the following studies were designed and executed as follows:

Specific aim 1 (Chapter 2): The aim of these studies was to investigate sound enamel resistance to demineralization, for which I compared SDF to SDF+KI, $AgNO_3$, KF, and DIW, using in vitro cariogenic challenges of varying sources and complexities. All enamel samples were randomly divided into five treatment groups: SDF, SDF+KI, $AgNO_3$, KF and DIW. Again, each group was randomly divided into two groups:

1. Demineralization using chemical model
2. Demineralization using biofilm model

Moreover, the goal was to understand the mechanism of applying KI after SDF solution on enamel, and to observe if it prevents staining.

Specific aim 2 (Chapter 3): The aim of these studies was to investigate the effectiveness of SDF and its individual components, Ag and fluoride ions, and SDF+KI in preventing enamel demineralization by using two different pH-cycling models (described by Featherstone et al. [1986]):

1. pH-cycling with fluoride intervention model
2. pH-cycling with placebo model

Moreover, the goal was to evaluate staining and caries prevention of SDF+KI.

Specific aim 3 (Chapter 4): The aim of these studies was to compare in-vitro remineralizing efficacy of SDF, SDF+KI, $AgNO_3$, KF and DIW, on artificial, early enamel caries lesions. Early enamel caries lesions were created, followed by treating specimens with SDF, SDF+KI, $AgNO_3$, KF, and DIW, and the extent of lesion remineralization was studied comprehensively in these scenarios:

1. Remineralization with mucin
2. Remineralization without mucin

Moreover, color assessment using a spectrophotometer was performed to evaluate the stain control ability of KI.

Significance

SDF has been demonstrated to be a safe, successful, and acceptable method to prevent and arrest dentin caries. SDF has also proven to be a suitable substitute for more expensive procedures in communities with limited resources or access-to-care barriers (Llodra, Rodriguez et al. 2005). It requires a simple procedure, which does not need expensive equipment, and more importantly, it is non-invasive, with a minimal risk of spreading infection. Since SDF is a valuable agent for caries prevention and management, understanding the anti-caries mechanism of SDF provides a better insight into practice innovation (Lo, Chu et al. 2001, Zhi, Lo et al. 2013).

SDF is currently being used in the arrestment of active dentin caries lesions. Little information exists about SDF's ability to prevent primary enamel caries. This research was therefore significant as it could lead to a more widespread use of SDF in caries prevention rather than just its management.

The only drawback regarding SDF application is the potentially irreversible black staining that it leaves on the tooth surface, which may not be acceptable and may cause patient dissatisfaction. Therefore, there was a need for more comprehensive and in-depth studies on the remineralization effects of SDF alone and in combination with KI, which has been shown to help reduce staining from SDF (Zhao, Mei et al. 2017). However, until my study, both staining level and anti-caries efficacy of SDF+KI had not been tested on enamel.

Thus, this research is significant because it provides high-quality evidence about the anti-caries efficacy of SDF treatment followed by KI, especially on enamel. Also, it may help an adult patient who is concerned about the staining issue of SDF (Horst, Ellenikiotis et al. 2016).

Innovation

The in-vivo and in-vitro studies on the ability of SDF to prevent caries had thus far been focused primarily on arresting dentin caries. Therefore, this study is innovative for using enamel specimens. Moreover, it is the first study to determine demineralization with both chemical and biofilm models as well as remineralization with and without protein (mucin). This may give more detailed results as to what happens in natural oral environments and show the specific effects of SDF, SDF+KI, fluoride and silver ion on enamel demineralization. Moreover, as for SDF staining issues**,** it was still unclear whether adding KI to SDF could change the anti-caries efficacy of SDF while improving staining issues.

CHAPTER 2: EFFECTIVENESS OF IN VITRO PRIMARY CORONAL CARIES PREVENTION WITH SILVER DIAMINE FLUORIDE - CHEMICAL VS BIOFILM MODELS

2.1. Introduction

Over the last 20 years, the high prevalence of dental caries and the increasing cost of healthcare pose a significant public health problem all over the world. In addition, treatment of dental caries in young, fearful, non-cooperative children or those with limited access to dental care or financial limitations can be challenging as untreated dental caries in children can cause pain, infections, and costly emergency room visits and/or hospitalizations. Moreover, current methods of early caries preventive treatment do not seem to successfully inhibit caries development (Blackburn, Morrisey et al. 2017, Oliveira, Rajendra et al. 2019). The current trend to manage the dental caries process aims to utilize minimally invasive, tissue-preserving, affordable and safe approaches, while efforts to create more effective anti-caries agents are still ongoing and desperately needed. Silver fluoride, or in stabilized forms such as silver diamine fluoride (SDF), has been used in Japan as early as the 1970s for both the treatment of dentinal hypersensitivity and dental caries and it has been rapidly implemented by dentists in the United States since 2015 (Horst, Ellenikiotis et al. 2016). Silver diamine fluoride ($Ag(NH_3)_2F$) is a colorless aqueous solution which contains both silver (Ag^+) and fluoride (F^-) ions. Silver is an antimicrobial agent which attacks cariogenic bacteria, promotes resistance to biofilm (re-)formation, while fluoride promotes remineralization of the tooth. SDF is a safe, minimally invasive approach which is effective and affordable

and might be helpful to those with special care needs and for lower income groups (Crystal and Niederman 2019, Johhnson and Serban 2019).

SDF has proven clinical efficacy for caries arrest on dentinal caries lesions in primary teeth (Gao, Zhang et al. 2016) and limited evidence indicates its effectiveness on caries arrest on permanent teeth (Llodra, Rodriguez et al. 2005) and there is also limited clinical evidence of SDF preventing new lesions formation. In vitro studies have shown that SDF prevents the formation of cariogenic biofilms (Zhao, Gao et al. 2018) including mono-species biofilms of *Streptococcus mutans* and *Actinomyces naeslundii*. SDF was also shown to prevent dentin demineralization (Mei, Li et al. 2013). SDF is currently being used in the arrestment of active dentin caries lesions. The major drawback associated with SDF is the permanent black staining that results in precipitation of silver ions on demineralized enamel. These silver ions precipitate as Ag_2S and react with organic material, leaving a black staining on the teeth which can be obvious depending on the location of the dental caries lesion (Crystal, Kreider et al. 2019). Therefore, a substantial barrier to widespread use of SDF is the patient/parental unwillingness to accept a permanent black staining (Crystal, Kreider et al. 2019, Karched, Ali et al. 2019). Based on Knight et al., a possible solution to minimize the staining issue is to apply saturated solution KI immediately after SDF application to bind free silver ions from SDF (Knight, McIntyre et al. 2006). KI reacts with free silver ions and forms a yellow precipitate of AgI, which is insoluble in water and prevents the black staining caused by SDF. However, no adequate in vitro data on the anti-caries efficacy of SDF and SDF+KI as a preventive agent in enamel could be retrieved. Moreover, the ability of SDF to prevent the demineralization of sound dental enamel; i.e., primary coronal caries

prevention, is yet to be investigated. Although permanent and primary enamel have some inherent differences (mineral composition, enamel rod density and overall thickness), the mechanisms of caries progression and remineralization are reported to be similar; therefore for the purpose of this study, I chose to use permanent enamel (Wilson and Beynon 1989, Wang, Tang et al. 2006, De Menezes Oliveira, Torres et al. 2010). The present laboratory study aimed to 1) evaluate the efficacy of SDF for caries prevention in enamel, 2) evaluate if applying KI after SDF affects its anti-caries efficacy while simultaneously retarding staining issues, and 3) compare chemical vs. biofilm models in inducing demineralization to study the differential efficacy of caries preventive agents. I hypothesized that a) SDF is an effective anti-caries agent in the inhibition of enamel demineralization, b) KI application immediately after SDF treatment can significantly reduce staining caused by SDF alone while not affecting SDF anti-caries efficacy, and c) SDF may be comparatively more effective in inhibiting demineralization in a biofilm model than in a chemical model.

2.2. Materials and Methods

Study Design

The study was determined to be exempt from IRB oversight IRB #: NS0911-07. The schematic of the experimental procedures in this study is shown in Figure 1. Briefly, 180 polished human permanent enamel specimens were assigned to five treatment groups after color and surface microhardness assessments: SDF, SDF+KI, $AgNO_3$, KF and DIW. Color assessment immediately after treatment application was performed only in the chemical model, as doing color assessment on the biofilm model samples would potentially add environmental bacteria to them during the measurement process.

Specimens were then demineralized using two different demineralization models – chemical and biofilm for five and three days, respectively. The biofilm isolated from the enamel blocks in the biofilm model was analyzed for Colony-Forming Units (CFU). All enamel samples were analyzed for changes in color and surface microhardness and using transverse microradiography (TMR) to determine integrated mineral loss and lesion depth.

Specimen selection and preparation

One hundred and eighty sound extracted human permanent teeth predominantly molars and premolars (anonymous donations from dental clinics) were used as specimens. Only buccal and/or lingual surfaces with no wear defects, fracture lines, or cracks were included in this study. Tooth crowns were cut into 4×4 mm specimens using a low-speed saw (Iso Met, Buehler, Lake Bluff, IL, USA). The teeth were stored in deionized water (DIW) containing thymol (0.1% w/v) during the sample preparation process. Specimens were ground and polished to create flat, planar parallel enamel surfaces using a Struers Rotopol 31/Rotoforce 4 polishing unit (Struers Inc., Cleveland, Pa., USA). The enamel specimens were serially ground using 1,200-, 2,400-, and 4,000-grit silicon carbide grinding paper. The specimens were then polished using a 1-μm diamond polishing suspension on a polishing cloth until the enamel surface had a minimum of a 3×4 mm highly polished facet across the specimen. The resulting specimens had a thickness range of 1.7–2.2 mm. (enamel and underlying dentin). The specimens were assessed under a Nikon SMZ 1500 stereomicroscope at 20× magnification for cracks, hypomineralized (white spot) areas, or other flaws in the enamel surface that would exclude them from use in the study. An experimental window, measuring approximately 2×4 mm, was created

on the human enamel specimens using acid-resistant, colored nail varnish (Sally Hansen Advanced Hard as Nails Nail Polish, USA), leaving sound enamel areas on either side. Specimens were stored at 100% relative humidity at 4° C until further use (Lippert, Churchley et al. 2015).

Pretreatment assessment

Sound enamel color assessment

Color assessments were performed by a single examiner to evaluate color changes among the treatment options. Commision Internationale de l'Eclairage (CIE) L* values were recorded. Measurements were performed using a spectrophotometer, Minolta Chroma meter CR-241 (Minolta Camera Co., Osaka, Japan) with D65 light against a white background. Calibration of the spectrophotometer was performed using a ceramic tile supplied by the manufacturer. The area of the specimens scored was a 3-mm diameter circle in the center of the enamel surface. All measurements were repeated three times (Alshara, Lippert et al. 2014).

Sound enamel surface microhardness

Specimens were assessed for sound enamel SMH using a microhardness tester (2100 HT; Wilson Instruments, Norwood, MA, USA). Each enamel specimen was secured on a 1-inch square acrylic block with sticky wax and then placed in the center of the hardness tester. Four baseline indentations spaced 100 μm apart were placed with a Vickers diamond under a 200 g load in the center of a flattened, polished sound enamel specimen, each with a dwelling time of 11 s. SMH was determined by measuring the indentation length using dedicated image analysis software (Clemex CMT HD version 6.0.011, Clemex Technologies Inc., Longueuil, Quebec, Canada).

SMH_{sound} was derived from the respective indentation lengths and recorded. Only specimens which fulfilled the criteria of $300 \leq SMH_{sound} \leq 400$ were acceptable for use in the study and were divided into groups for each treatment and intervention group within each study.

Specimen stratification

The enamel specimens were stratified into two study groups with 18 specimens per study group to ensure that there were no significant differences in SMH_{sound} between treatment and study groups.

Biofilm model pretreatment disinfection of the enamel blocks

In order to avoid contamination and the growth of environmental microbes, samples were dipped into 70% alcohol for 2 seconds and air dried for 15 min before applying the treatment solutions.

Treatment groups

Enamel specimens were randomized into five treatment groups of 36 specimens each: SDF, SDF+KI, $AgNO_3$, KF and DIW (placebo groups).

- SDF: 38% SDF (Advantage Arrest, Elevate Oral Care LLC, Fl, USA) solution; nominally 253,900 ppm Ag^+; 44,800 ppm F^-
- SDF+KI: SDF application followed by supersaturated KI application (Potassium iodide 39% w/v solution, 30315, Sigma–Aldrich, St. Louis, Mo, US)
- $AgNO_3$: silver control; 253,900 ppm Ag^+ (Silver nitrate 31630, Sigma–Aldrich)
- KF: fluoride control; 44,800 ppm F^- (Potassium fluoride 60238, Sigma–Aldrich)
- DIW: negative control

A micro applicator (Regular; Premium Plus International Ltd., Hong Kong, China) was used to apply SDF solution. All other solutions were applied to the specimen's enamel surface with a micro-brush (Premium Plus Regular Tip Micro A microbrush). All solutions were left on the enamel surface undisturbed for 60 min before color assessment. For the SDF+KI group, SDF was applied immediately followed by a saturated KI solution until the creamy yellow solution turned clear, and the reaction products were wiped off using sterile cotton swabs.

Post-treatment color assessment

In the chemical model, color assessments were performed again, after application of the interventions. In the biofilm model, in order to avoid contamination of the specimen with environmental bacteria, color assessments were not performed between baseline and after treatment. L* was recorded for each specimen and the following variable was calculated: $\Delta L^* = L^*_{post} - L^*_{sound}$. All measurements were repeated three times.

Demineralization using chemical model

Immediately after color measurements, early caries lesions were created in the specimens utilizing a modified demineralization protocol based on the White (1987) protocol (White 1988). Artificial lesions were formed in the enamel specimens by a 5-day immersion in a solution containing 0.1 M lactic acid, 4.1 mM $CaCl_2 \times 2\ H_2O$, 8.0 mM KH_2PO_4 (all Sigma-Aldrich) and 0.2% w/v Carbopol 907 (BF Goodrich Co., USA), pH adjusted to 5.0 using KOH, at 37°C [18]. The protocol of a 5-day immersion was based on pilot data. Demineralization was performed at a ratio of 10 ml of solution per specimen. After lesion creation, specimens were rinsed with DIW and stored at 100 % relative humidity at 4°C.

Demineralization using biofilm model

The blocks (not the enamel surface) were disinfected using 70% ethanol wipe, and then they were kept under the UV light for 15 min. After specimen preparation was completed, specimens were mounted on the lid of a six-well plate (Fisher Scientific Co., Silver Spring, Md.) with acrylic cubes. Specimens were demineralized by aerobic incubation in a clinically relevant overnight culture of cariogenic bacteria including *S. mutans*. An overnight culture of multi-species bacterial mix, which was previously collected from human saliva under IRB approval #1406440799 based on Ayoub et al. 2019 (Ayoub, Gregory et al. 2019), was mixed with an overnight culture of *S. mutans* strain UA159 (ATCC 700610) in a 10:1 volume ratio. Each specimen was incubated in a six-well tissue culture plate containing the bacterial inoculum for 72 h, aerobically to create caries lesions. This time period was chosen to achieve a similar level of demineralization as the chemical model. The media and the plates were changed daily. The growth media contained Brain Heart Infusion (BHI) supplemented with 0.2% sucrose. This experiment was repeated three times with six samples per group (totaling n = 18 per intervention group).

Post-intervention assessment

Colony counting

Isolated biofilm was analyzed for bacterial viability using an established method (Zhang, He et al. 2015). For CFU counting, biofilm on the exposed surfaces of the enamel blocks was wiped off with a micro brush. The tip of the micro brush was placed in 1 ml of saline and sonicated. One hundred µl of the biofilm suspension was spread with a sterile glass rod on blood agar plates and incubated for 48 h in aerobic conditions at 37°C. Finally, the

colonies on the plates were counted to calculate CFU/ml. Again, after lesion creation, enamel specimens were rinsed with DIW, and kept at 100 % relative humidity at 4°C until future analysis.

Post-intervention color assessment

In both chemical and biofilm models, color assessments were performed after demineralization. L* was recorded for each specimen and the following variable was calculated: $\Delta L^*_{intervention} = L^*_{intervention} - L^*_{sound}$. All measurements were repeated three times.

Surface microhardness change

After completion of the studies, all specimens were again subjected to surface microhardness measurements as described above. A second set of four indentations was placed on each specimen in close proximity and to the right of the baseline indentations, yielding SMH_{post}. The extent of percent change in SMH for each individual specimen was calculated as follows: $\%SMHchange = 100*(SMH_{sound} - SMH_{post})/SMH_{sound}$.

Transverse Microradiography

One section per specimen, approximately 100 μm in thickness, was cut from the center of each specimen and across the lesion window and sound enamel areas using a Silverstone-Taylor Hard Tissue Microtome (Scientific Fabrications Laboratories, USA). The sections were placed in the TMR-D system and X-rayed at 45 kV and 45 mA at a fixed distance for 12 s. An aluminum step wedge was also X-rayed under identical conditions. The digital images were analyzed using the TMR software v.3.0.0.18. A window (approximately 400 × 400 μm), representative of the entire lesion area and not containing any cracks, debris or other alterations, was selected for analysis. Sound enamel mineral

content was assumed to be 85% v/v. The following variables were recorded for each specimen/section: ΔZ - integrated mineral loss: (product of lesion depth and the mineral loss over that depth), L - lesion depth.

Statistical Analysis

With a sample size of 18 specimens per group in each part of the study, the study has 80% power to detect a difference of 10% for %SMHchange, 15% for ΔZ, and 27% for Land 27% for CFU. The calculations assume two-sided tests conducted at a 5% significance level for each type of comparison, with coefficients of variance estimated at 0.1 for %SMHchange, 0.15 for ΔZ, and 0.27 L and 0.27 for CFU.

Separate analyses were performed for biofilm and chemical models. VHN hardness (the percent change in surface microhardness), mineral loss, lesion depth, log-transformed CFU and color changes (ΔL*) were analyzed using one-way ANOVA to examine the effect of treatment types. Experiment units were included in the model as a random effect. All pair-wise comparisons from ANOVA analysis were made using Fisher's Protected Least Significant Differences to control the overall significance level at 5%. Analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

2.3. Results

Microhardness

The %SMHchange data for both models are shown in Figure 2 and Figure 3. In the chemical model, there were no statistically significant differences between SDF and SDF+KI ($p=0.0515$) in preventing enamel demineralization. There were statistically significant differences between SDF and SDF+KI in preventing caries lesion formation compared to KF, $AgNO_3$ and DIW (all $p<0.0001$). $AgNO_3$ and DIW exhibited a

significant reduction in their VHN values compared to KF (both $p<0.0001$). There was no difference between $AgNO_3$ and DIW ($p=0.1756$). In the biofilm model, there were statistically significant differences in preventing caries lesion formation between SDF and SDF+KI ($p<0.0001$). SDF+KI, $AgNO_3$ and DIW exhibited a significant reduction in their VHN values compared to KF (all $p<0.0001$). There was no difference between SDF and KF ($p=0.0690$). There was also no difference between $AgNO_3$ and DIW ($p=0.2380$).

TMR

The ΔZ and L data for both models and all treatment groups can be found in Table 1. In the chemical model, there was a significant difference between SDF, SDF+KI, and KF in demineralization inhibition compared to $AgNO_3$ and DIW ($p <0.0001$). In the biofilm model, for both ΔZ and L, there were no statistically significant differences between any of the treatment groups ($p=0.0750$ and $p=0.1659$, respectively).

CFU

CFU/ml values for the biofilm model are shown in Figure 4. There was a significant difference between SDF and SDF+KI in inhibiting *S. mutans* and other salivary bacteria compared to $AgNO_3$ and DIW ($p<0.0001$). There were no differences between KF, $AgNO_3$ and DIW ($p\geq0.07$).

Color Assessment

ΔL*data for both models and all treatment groups are shown in Figure 5 and Figure 6. ΔL* values were evaluated for after treatment change from baseline and post-intervention change from baseline in the chemical model, as well as post-intervention change from baseline in the biofilm model. In both chemical and biofilm models, L* values from baseline to post intervention demonstrate applying KI after SDF significantly

reduced the dark staining caused by SDF ($p < 0.0001$). Accordingly, SDF+KI groups had significantly higher ΔL^* values than SDF alone, whereas group SDF and $AgNO_3$ groups presented significantly lower ΔL^* compared with SDF+KI groups.

2.4. Discussion

To the authors' knowledge, this is the first study that investigated the ability of SDF to prevent enamel demineralization while utilizing both chemical and biofilm models. The present research is therefore significant, as understanding the mechanism behind SDF could lead to more widespread use of SDF in primary coronal caries prevention. Appropriate silver ($AgNO_3$) and fluoride (KF) controls as well as two models of demineralization, chemical and biofilm, were included to elucidate the mode of action of SDF. Furthermore, KI was investigated as a post-SDF application treatment to mitigate staining associated with SDF.

A distinct difference in the comparative efficacy of SDF vs. SDF+KI was noted between the chemical and biofilm models. While both were equally and more effective than all other interventions in preventing enamel demineralization in the chemical model, this was not the case in the biofilm model. Here, KI impaired the efficacy of SDF. These results were in agreement with previous studies on dentin (Knight, McIntyre et al. 2009, Mei, Li et al. 2013, Hamama, Yiu et al. 2015). There are several possible explanations for the present observations:

1) KI may reduce silver ion bioavailability, thus the silver ions are not able to bind with and kill bacteria (anti-bacterial effect of silver). Silver ions are assumed to be primarily responsible for the antimicrobial action of SDF by inhibiting the growth of *S. mutans*, a primary pathogen in dental caries. Thus *S. mutans* is less able to

form a biofilm on teeth treated with SDF ex vivo. While excess silver ions are removed by KI in both demineralization models, the impact in the chemical model is negligible as silver ions do not appear to interact in de- and remineralization processes (Yu, Zhao et al. 2018).

2) A second hypothesis for this data is that KI increases the organic acid production of bacteria, which in turn causes increased demineralization of tooth structures.
3) Another hypothesis is that the combination of SDF+KI may promote bacterial enzymes involved in carbohydrate metabolism and sugar uptake.

However, the results of a biofilm study employing dentin specimens was in agreement with the results of the chemical model of this study in that SDF+KI was as effective as SDF alone against dental caries (Knight, McIntyre et al. 2005). In that study optical density was used to determine the level of bacterial growth, and no data were provided to show correlations between optical density readings and concentrations of *S. mutans* in the solution. To the authors' knowledge no biofilm studies on SDF+KI have been conducted on sound enamel which highlights the novelty of this research.

In both models, SDF and SDF+KI were superior in their ability to prevent caries lesion formation than $AgNO_3$ and DIW. SDF was more effective than KF in both biofilm and chemical models; however, this difference was not significant in the biofilm model. This discrepancy between models can be due to a host of reasons including the interaction of the biofilm with the enamel surface, different degrees of attachment of the biofilm, biofilm growth and acid production. Consequently, this leads to a pH gradient within the biofilm, which is not comparable to how demineralization occurred in the chemical model. Moreover, in the biofilm model media and plates were changed daily

which may cause the biofilm and/or some of the treatments (SDF and KF) to be removed in some group/specimens more than others during transfer.

Topical application of $AgNO_3$ solution had little to no effect in both models and there was no difference between $AgNO_3$ and DIW in both models. It has been shown previously that $AgNO_3$ is washed away if it is applied without a protective layer of fluoride varnish after $AgNO_3$ application (Zhao, Mei et al. 2017).

Based on the VHN results of the chemical model, SDF inhibits demineralization more effectively than KF and $AgNO_3$ alone. Accordingly, it can be assumed that synergistic effects between silver and fluoride exist. However, this assumption was not supported by the biofilm model results as there was no difference between SDF and KF as discussed earlier.

The TMR data for the chemical model were in agreement with the VHN data. However, this was not the case for the biofilm model. While TMR is considered the gold standard technique for quantifying (changes in) mineral loss and lesion depth of caries lesions (Ten Bosch and Angmar-Mansson 1991), it does lack sensitivity in accurately assessing the mineral status of early lesions. Due to the lesser overall extent of demineralization in the biofilm in comparison to the chemical model, the present findings highlight the need to employ several, complementary analytical techniques.

SDF was shown to prevent multi-species cariogenic biofilm growth. The biofilm data (Fig. 4) indicated that growth inhibition of *S. mutans* and other salivary bacteria was higher with SDF alone than with SDF + KI which supports the VHN data. However, $AgNO_3$ did not provide antimicrobial benefits. Destruction of the outer bacterial cell membrane and cytoplasmic extrusion is due to the high reactivity of silver ions to the

bacterial enzymes that contain sulfur and phosphorus components in the bacterial cell wall, including the phosphoenolpyruvate phosphotransferase system, which transfers sugars through the cell membrane. This high reactivity is due to the difference in charges between the negatively charged bacterial cell wall and the positively charged silver ions which result in an electrostatic adhesion between the bacterial enzymes and the silver particles. Electrostatic adhesion of silver ions with bacterial enzymes inactivates them and prevents metabolic activities of the bacterial enzymes via silver-induced protein coagulation (Hamama, Yiu et al. 2015, Ishiguro, Mayanagi et al. 2019). In addition to the effect of ionic silver, fluoride which is the other component of SDF, is the most effective and widely used anti-caries agent found in both self- and professional products. Primarily, fluoride decreases the rate of enamel demineralization and enhances remineralization of enamel caries lesions, which is the main mode of action of fluoride (Lippert, Newby et al. 2009). Fluoride inhibits demineralization by being absorbed onto the hydroxyapatite crystals on the tooth surface. Fluoride also promotes remineralization of tooth mineral hydroxyapatite, and by incorporation of fluoride into the remineralized structure, it thus makes it more resilient to a repeated acid attack (Crystal, Marghalani et al. 2017). Furthermore, fluoride has also been shown to prevent the formation of cariogenic biofilms, via binding to bacterial cellular components and influence enzymes which effectively prevent the carbohydrate metabolism of acidogenic oral bacteria and their sugar uptake (Mei, Li et al. 2013). Presently, however, there was only a mild antimicrobial fluoride effect as KF did not prevent biofilm growth as effectively as SDF (Figure 4).

The color measurements utilizing a spectrophotometer were performed to determine changes in the CIE Lab color space of the enamel specimens. The biggest disadvantage of the use of SDF is the dark staining of the tooth surface. Consequently, Ag_3PO_4, AgO_2 and AgS_2 compounds, found in SDF-treated caries lesions, turn Ag^+ to metallic silver nanoparticles which after light exposure causes the caries lesions to turn black. This may impact SDF acceptance as a treatment option (Crystal, Kreider et al. 2019, Crystal and Niederman 2019, Li, Liu et al. 2019). The results demonstrated that KI helped reverse dark staining caused by SDF in the chemical model immediately after application. This was in agreement with the outcomes of other studies performed on dentin (Gupta, Thomas et al. 2019, Zhao, Chu et al. 2019). However, KI was not able to permanently prevent SDF-related staining after demineralization (Figure 5), which was also observed on dentin previously (Zhao et al, 2017). Inability of KI in completely removing the discoloration caused by SDF may be due to high photosensitivity of AgI which can dissociate into metallic silver and iodine by exposure to light. Likewise, there may have been an insufficient amount of KI which led to an excess of free Ag^+ (Zhao, Mei et al. 2017). Not surprisingly, the DIW group exhibited significantly more whiteness than all other groups in the chemical model due to the formation of an early white spot lesion.

Several limitations need to be highlighted. This laboratory model did not include remineralization periods. The effects of SDF and SDF+KI under chemical and/or bacterial pH-cycling models should be conducted to better understand the efficacy of SDF compared to SDF+KI and its individual components. Furthermore, SDF was only applied once in this study. It has been shown that a single application of SDF is

inadequate for constant caries inhibition effects especially on sound enamel. Biannual application for the duration of two years has been recommended to increase the chance of sustained caries arrest, and it may be the same to prevent new lesions (Crystal, Rabieh et al. 2019). Moreover, only the immediate effect of SDF was studied presently but not its ability to longitudinally prevent caries. It is noteworthy to mention that the single species *S. mutans* along with multi-species bacteria from human saliva were used. While this is somewhat removed from the complexity of oral biofilms, the key advantage of this biofilm model was that the bacterial cell growth was reliable and comparable among the different treatment groups along with the diversity in the bacterial species (Savas, Kucukyilmaz et al. 2015, Yu, Mei et al. 2018). Lastly, based on the results of this study it is recommended to analyze lactic acid production in future studies to verify whether applying KI can cause an increase in acid production or not (Liu, Lo et al. 2012).

Within the limitations of the study, SDF may offer an alternative biological approach in preventing primary coronal caries in the future. KI application after SDF significantly improved the dark staining and helped enhance the esthetic outcome by stain reduction. The results from the chemical model show that KI application did not impair the anti-caries efficacy of SDF. However, in the biofilm model, KI diminished the anti-caries efficacy of SDF. Further studies are granted to corroborate whether these effects are sustained using clinical models.

Under the conditions of this study, SDF appears to be an effective antibacterial and anti-caries topical agent that has the potential to prevent enamel caries. While KI application immediately after SDF treatment can substantially reduce the discoloration

caused by SDF, KI impairs SDF's ability to prevent biofilm-mediated demineralization. Further research using clinical models would be needed to establish conclusive evidence.

Figure 1. Schematic of the experimental procedures

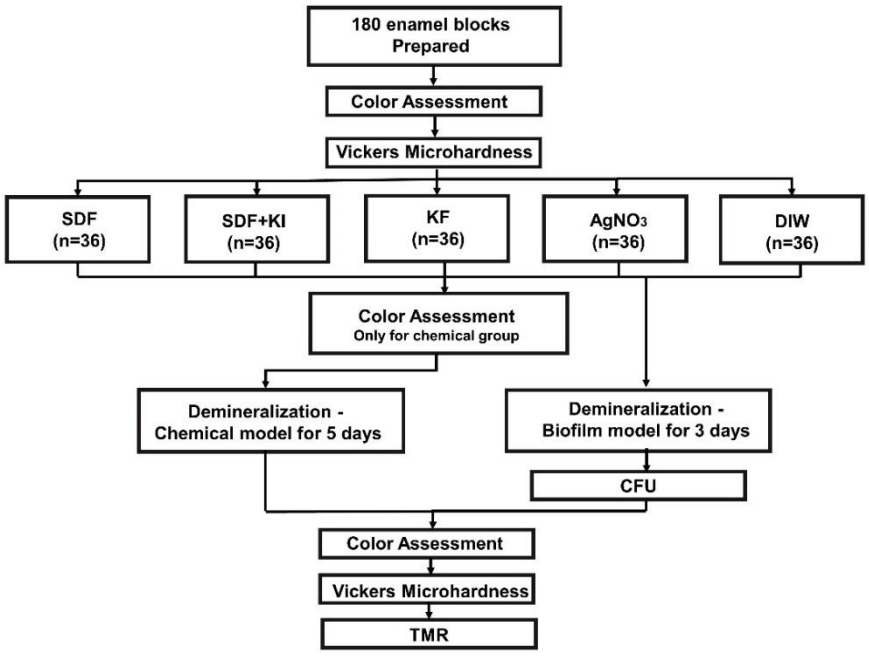

Figure 2. Chemical model - percent change in surface microhardness (%SMH change) from baseline

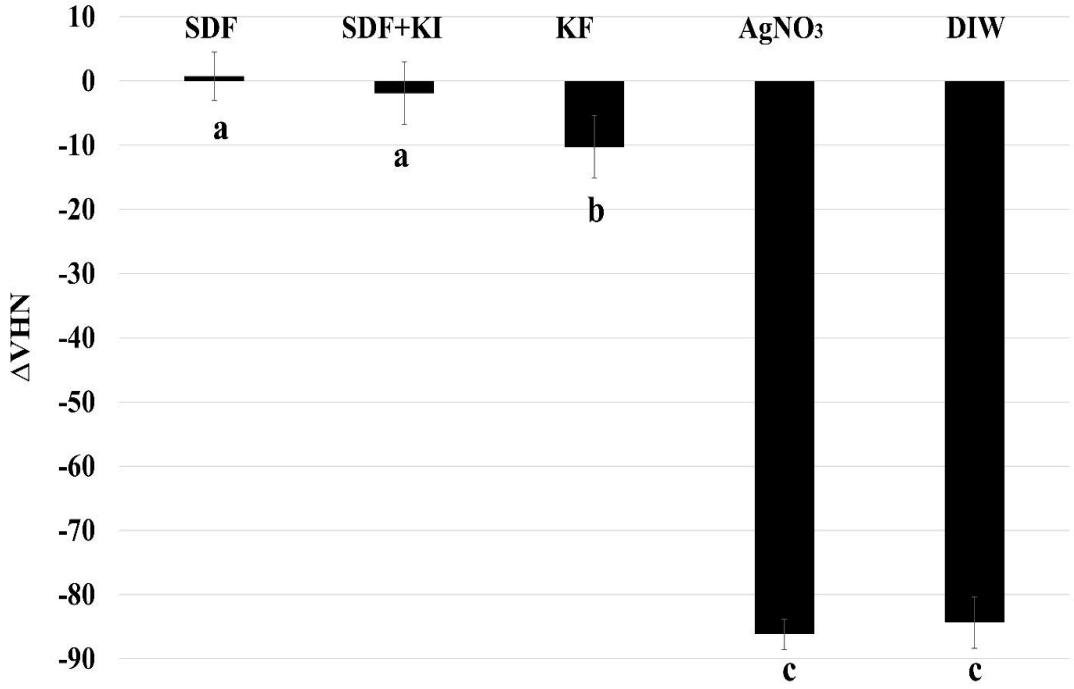

Figure 3. Biofilm model - percent change in surface microhardness (%SMH change) from baseline

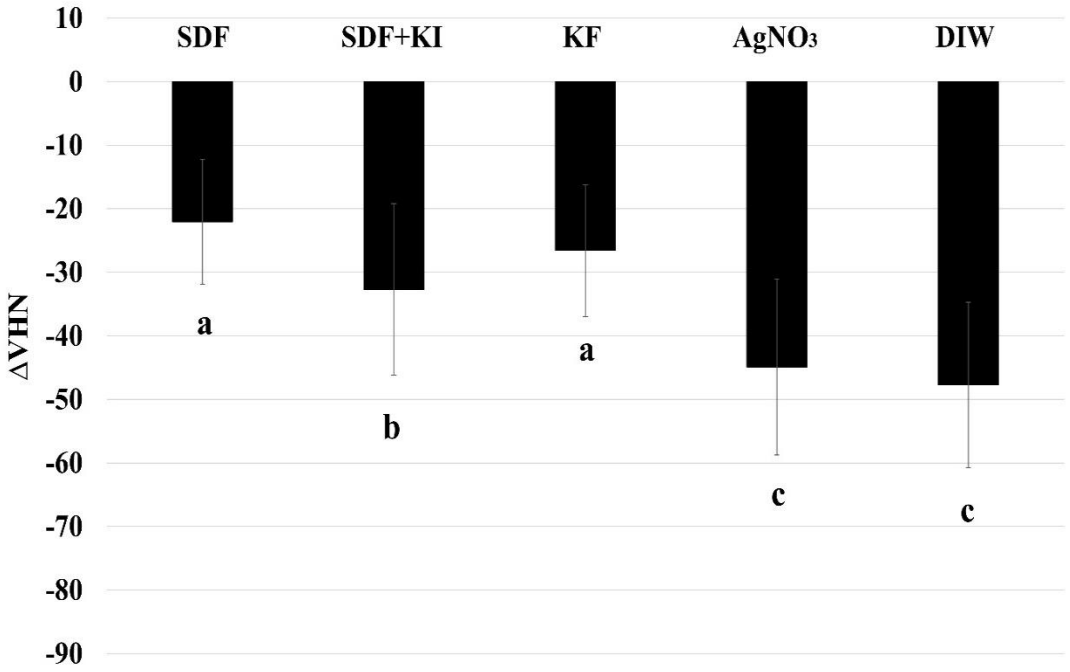

Figure 4. Biofilm model - changes in colony-forming units (Log CFU/ml) from baseline

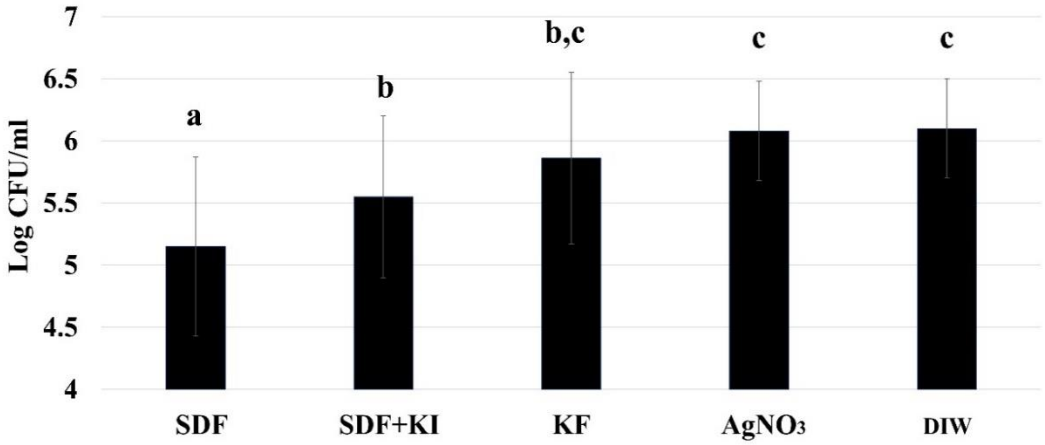

Figure 5. Chemical model - color change (ΔL*)

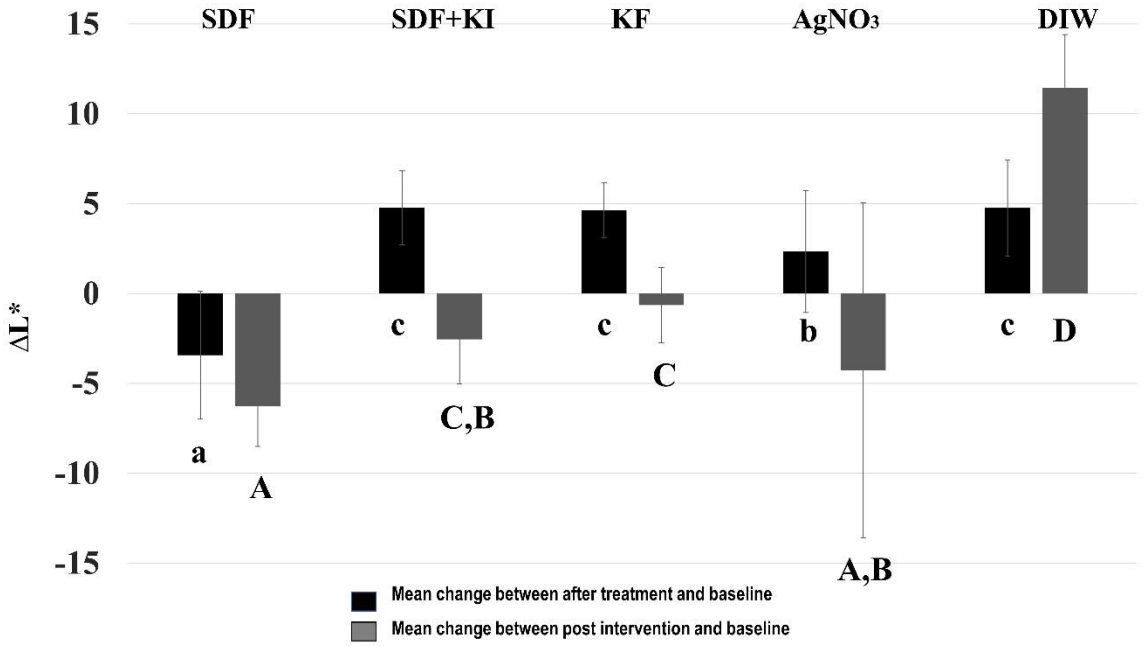

Figure 6. Biofilm model - color change (ΔL*)

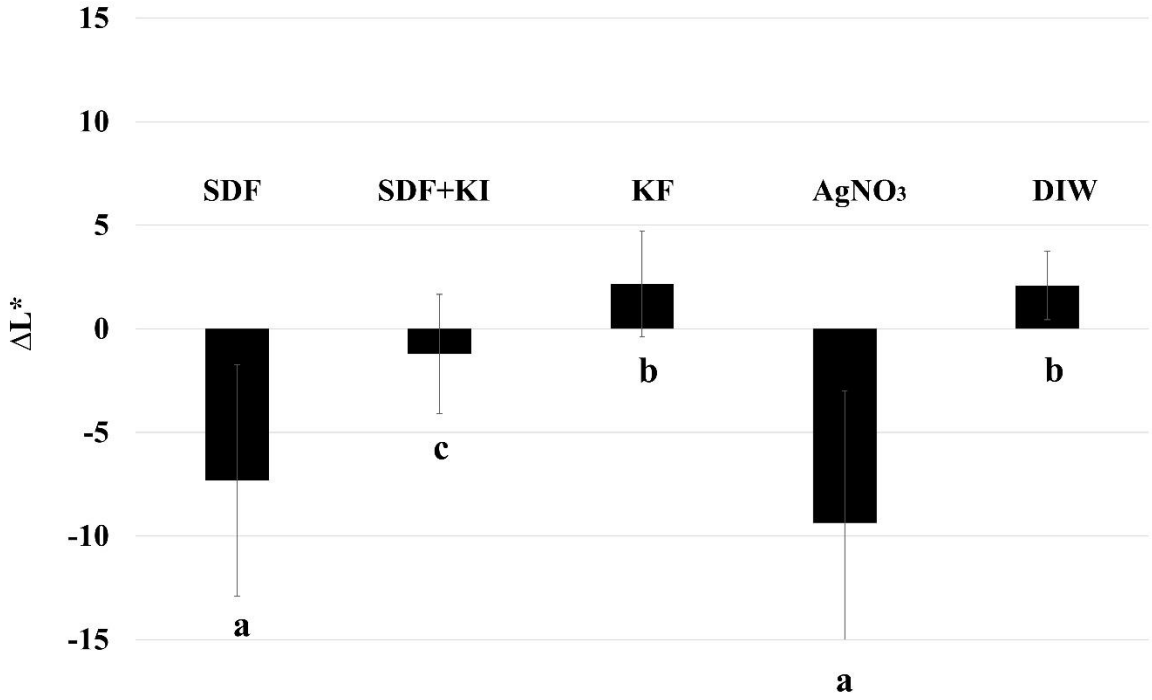

Table 1. TMR data (all means ± standard deviations) for both models

	Chemical model		Biofilm model	
Intervention	ΔZ (vol%min×µm)	L (µm)	ΔZ (vol%min×µm)	L (µm)
SDF	96 ± 94	6 ± 7	279 ± 175	16 ± 14
SDF+KI	124 ± 99	6 ± 7	323 ± 221	12 ± 11
KF	198 ± 210	9 ± 10	218 ± 228	12 ± 13
$AgNO_3$	1390 ± 497	49 ± 13	343 ± 166	17 ± 8
DIW	1334 ± 478	52 ± 12	407 ± 181	20 ± 8

Figure legends

Figure 1. Schematic of the experimental procedures.

Figure 2. Chemical model - percent change in surface microhardness (%SMH change; mean ± standard deviation) from baseline. Different letters highlight statistically significant differences between treatment groups.

Figure 3. Biofilm model - percent change in surface microhardness (%SMH change; mean ± standard deviation) from baseline. Different letters highlight statistically significant differences between treatment groups.

Figure 4. Biofilm model - changes in colony-forming units (Log CFU/ml; mean ± standard deviation) from baseline. Different letters highlight statistically significant differences between treatment groups.

Figure 5. Chemical model - color change (ΔL*; mean ± standard deviation). Black bars: mean change between after treatment and baseline-lower case letter for black bars shows after treatment change from baseline; gray bars: mean change between post intervention and baseline-upper case letter for gray bars shows post-intervention change from

baseline. Different letters highlight statistically significant differences between treatment groups.

Figure 6. Biofilm model - color change (ΔL*; mean ± standard deviation). Black bars: mean change between post intervention and baseline. Different letters highlight statistically significant differences between treatment groups.

CHAPTER 3: THE EFFECT OF SILVER DIAMINE FLUORIDE IN PREVENTING IN VITRO PRIMARY CORONAL CARIES UNDER PH-CYCLING CONDITIONS

3.1. Introduction

Dental caries continues to be one of the most prevalent preventable diseases in the US and worldwide (Kassebaum, Smith et al. 2017), with most cases occurring among populations with low-income and ethnic/racial minority backgrounds (Dye, Thornton-Evans et al. 2015). Current methods of prevention like fluoride varnish applications and sealants, although effective to a large extent, are difficult and costly to implement on a large scale basis in populations who need them the most (Griffin, Wei et al. 2016).

Silver diamine fluoride (SDF) has been rapidly adopted as an agent for caries arrest in dentin caries lesions (Crystal, Janal et al. 2020). Its use as an agent for caries prevention has been studied to a lesser extent, and it is described as a simple and low-cost method that does not require the complex training of the health professional or the cooperation of the patient. This approach may be of great utility as an alternative to more costly preventive methods in communities with limited resources (Llodra, Rodriguez et al. 2005).

Remineralization of dental caries lesions and prevention of demineralization at the earliest phase have gained recognition in the minimally invasive approach to dental caries treatment in recent years (Zhi, Lo et al. 2012, Dorri, Martinez-Zapata et al. 2017, Gao, Zhao et al. 2018). However, current methods of early preventive care are often insufficient to prevent new caries lesions in high risk individuals, (Featherstone and Doméjean 2012, Featherstone, Fontana et al. 2018) which suggests the need for innovative and alternative preventative approaches for the management and prevention of

dental caries that are minimally invasive and less traumatic in children (Selwitz, Ismail et al. 2007).

SDF is a topical solution comprised of silver, ammonia and fluoride ($Ag(NH_3)_2F$). It is a safe, effective, efficient, noninvasive and cost-effective method in caries management (Timms, Sumner et al. 2020). The antibacterial properties of silver in addition to the remineralization promotion ability of fluoride act together to both prevent the progression and to arrest dental caries lesions (Mei, Lo et al. 2018, Timms, Sumner et al. 2020). Several randomized clinical trials support its use primarily for the treatment of dentin caries, with few studies highlighting its potential usefulness in preventing caries lesion formation (Chu, Lo et al. 2002, Llodra, Rodriguez et al. 2005, Gao, Zhao et al. 2016). In addition, there is currently no clinical or in-vitro evidence supporting the use of SDF as a preventative agent on sound enamel. The most significant adverse effect of SDF is non-medical and is the permanent dark staining of the lesion where SDF is applied. This is a major challenge (depending on the visibility of the caries lesions) in the acceptance of SDF as a treatment option for some patients/parents (Crystal, Kreider et al. 2019, Karched, Ali et al. 2019).

Potassium iodide (KI) has been reported and recommended to reduce SDF's dark staining by reacting with free silver ions to form a yellow precipitate of silver iodide (Knight, McIntyre et al. 2006). Nevertheless, there is conflicting evidence about the effectiveness of KI in preventing dark staining without impacting the prevention and arrest of dental caries (Seifo, Robertson et al. 2020, Sorkhdini, Gregory et al. 2020). pH cycling models are rapid, repeatable, cost effective, and have a higher level of scientific control, and sensitivity to response variables compared to clinical models which

makes them ideal to test and evaluate the efficacy of new products (Lobo, Goncalves et al. 2005, Buzalaf, Hannas et al. 2010). In addition, pH-cycling models have been validated to evaluate the dose-response effect of fluoride on enamel and early enamel caries lesions (Featherstone, Stookey et al. 2011). For these reasons, the pH-cycling model based on that described by Featherstone et al. (2011) (Featherstone, Stookey et al. 2011) were used in this study to determine, 1) the efficacy of SDF in preventing enamel caries lesion formation under pH cycling conditions in the presence or absence of twice-daily fluoride or placebo treatments, and 2) to evaluate staining and caries prevention of SDF+KI.

I hypothesized that a) SDF is still effective in enamel caries prevention with twice-daily fluoride application, and b) applying KI after SDF application can mitigate dark staining and at the same time does not negatively affect the anti-caries ability of SDF.

3.2. Materials and Methods

Study Design

The study was exempted from Institutional Review Board (IRB) supervision IRB #: NS0911-07. The flow chart of the study is shown in Figure 7. One hundred and eighty polished human permanent enamel samples were allocated to five treatment groups after color and surface microhardness assessments: SDF, SDF+ Potassium iodide (KI), Silver nitrate ($AgNO_3$), Potassium fluoride (KF), and deionized water. The study performed color assessment immediately after the treatment application. Enamel samples were then randomized into two pH-cycling groups: 1- pH-cycling with fluoride intervention and 2- pH-cycling with placebo. Specimens were pH cycled for 7 days. All specimens were then

evaluated for changes in color using a colorimeter, Vickers surface microhardness and utilizing transverse microradiography to measure integrated mineral loss and lesion depth.

Specimen selection and preparation

This experiment used one hundred and eighty sound extracted human permanent teeth (buccal and/or lingual surfaces of predominantly molars and premolars from dental clinics' anonymous donations were used) with no wear, cracks, or defects. Each tooth was cut into 4×4 mm specimens, then ground and polished to create flat specimens, as previously descried (Sorkhdini, Gregory et al. 2020). Nikon SMZ 1500 stereomicroscope at 20× magnification was used to check the enamel samples with a thickness range of 1.7–2.2 mm for flaws. The percentage of specimen losses during preparation was 30%, and no specimen was lost during the experimental procedure. The final samples were kept at 100% relative humidity at 4° C.

Pretreatment assessment

Sound enamel color assessment

Changes in color were measured using a spectrophotometer, as described previously, using Minolta Chroma meter CR-241 (Minolta Camera Co., Osaka, Japan) with D65 light against a white background (Alshara, Lippert et al. 2014). Commision Internationale de l'Eclairage (CIE) L* values were recorded, and all measurements were repeated three times.

Sound enamel surface microhardness (SMH)

Specimens were evaluated for sound enamel surface microhardness utilizing a microhardness tester as described previously (2100 HT; Wilson Instruments, Norwood,

MA, USA) (Sorkhdini, Gregory et al. 2020). Image analysis software (Clemex CMT HD version 6.0.011, Clemex Technologies Inc., Longueuil, Quebec, Canada) was used to determine the indentation length of the SMHsound. Enamel samples which fulfilled the criteria of $300 \leq$ SMHsound ≤ 400 were used in this study.

Specimen stratification

Between treatment and study groups, one hundred and eighty enamel specimens were stratified into five treatment groups with 36 specimens per treatment group to ensure that there were no significant differences in SMHsound.

Treatment groups

Enamel samples were randomized into five treatment groups of 36 samples each: SDF, SDF+KI, $AgNO_3$, KF and deionized water (placebo).

- SDF: 38% SDF (Advantage Arrest, Elevate Oral Care LLC, Florida, USA) solution; nominally 253,900 ppm Ag; 44,800 ppm F (Advantage Arrest, Fl, USA)
- SDF+KI: SDF application followed by supersaturated KI application (Potassium iodide 39% w/v solution, 30315, Sigma–Aldrich, St. Louis, US)
- $AgNO_3$: silver control; 253,900 ppm Ag (Silver nitrate 31630, Sigma–Aldrich, St. Louis, USA)
- KF: fluoride control; 44,800 ppm F (Potassium fluoride 60238, Sigma–Aldrich, St. Louis, USA)
- Deionized water: negative control

A micro applicator (Regular; Premium Plus International Ltd., Hong Kong, China) was used to apply SDF solution to the enamel surface. KI, KF, $AgNO_3$ and deionized water were applied using a micro-brush (Premium Plus Regular Tip Micro A microbrush). For

the SDF+KI group, after SDF application saturated KI solution was applied immediately until the creamy yellow solution turned clear (Zhao, Mei et al. 2017). All solutions were left on the enamel surface undisturbed for 60 min.

Post-treatment color assessment

After the 60-minute treatment the reaction products were rubbed off using sterile cotton swabs. Immediately afterwards, change in color was assessed again as described above**.** L* was recorded for each sample and ΔL* calculated as follows: $\Delta L^* = L^*_{post} - L^*_{sound}$.

pH-cycling

Immediately after color measurements, 36 enamel specimens from each treatment group were allocated to two intervention groups to ensure no significant differences in SMH sound:

1- pH-cycling with fluoride intervention model: pH-cycling with fluoride solutions containing 275 ppm fluoride. The fluoride concentration corresponds to the dilution (1:3) of dentifrices containing 1100 ppm F in the oral cavity during toothbrushing. The fluoridated solution was prepared with NaF (Sodium fluoride, 97%, extra pure, 191270250, Acros Organics, New Jersey, USA) and deionized water.

2- pH-cycling with placebo model: deionized water (i.e. fluoride-free placebo) as a control

Eighteen blocks from each treatment group were submitted for five days of pH-cycling utilizing a protocol based on that by Featherstone et al. (2011) (Featherstone, Stookey et al. 2011), followed by two days of storage in a remineralizing solution. The blocks were kept individually in a demineralizing solution (2.0 mM calcium, 2.0 mM phosphate, 0.030 ppm F, in 75 mM Acetic Acid, pH 4.3) for 3 h and in a remineralizing solution (1.5

mM calcium, 0.9 mM phosphate, 150 mM of KCl, 0.050 ppm F^- in 20 mM cacodylic buffer, pH 7.4) for 21 h. Twice a day (before and after immersion in the demineralizing solution), the blocks were washed with deionized water and subjected to one-minute immersion in fluoride or deionized water treatments. This cycle was repeated for five days and the enamel blocks then remained in the remineralizing solution for two days. All treatments were performed at a ratio of 10 ml of solution per specimen and all solutions were renewed prior to the start of each treatment. All specimens were washed with deionized water before and after each immersion in the solutions. The remineralization and demineralization were carried out in the incubator at 37ºC. After completion of the pH cycling phase, all specimens were rinsed with deionized water and stored at 100 % relative humidity at 4°C.

Post-intervention assessment

Post-intervention color assessment

Change in color was assessed on all samples after pH cycling as described above. L* was recorded for each sample and this variable was calculated: $\Delta L^*_{intervention} = L^*_{intervention} - L^*_{sound}$. All the color assessment measurements were conducted three times, and the mean was reported.

Surface microhardness change

All samples underwent surface microhardness assessment as described above. On the right of the baseline indentations, a second set of four indentations was inserted on each sample, yielding SMHpost. The percent change in surface microhardness for each sample was determined as follows: %SMHchange = 100 × (SMHsound - SMHpost)/SMHsound.

Transverse Microradiography

One section per samples, approximately 100 µm in thickness, was cut from the center of each sample and across the lesion window and sound enamel areas using a Silverstone-Taylor Hard Tissue Microtome (Scientific Fabrications Laboratories, USA) as described previously (Sorkhdini, Gregory et al. 2020). ΔZ - integrated mineral loss (product of lesion depth and the mineral loss over that depth), L - lesion depth were documented for each sample section.

Statistical Analysis

The study had 80% power to detect a difference of 10% for %SMHchange, 15% for ΔZ, 27% for L and 15% for color changes (ΔL*), with an overall sample size of 18 samples per group. The calculations assumed two-sided tests performed at a 5% significance level for each type of comparison, with coefficients of variance estimated at 0.1 for %SMHchange, 0.15 for ΔZ, 0.27 for L and 0.15 for color changes (ΔL*). %SMHchange, ΔZ, L, and color changes were evaluated using two-way ANOVA to assess the efficacy of different types of treatments and models, as well as interactions between treatment types and models. All pair-wise comparisons from ANOVA analysis were produced using Fisher's Protected Least Significant Differences to control the overall significance level at 5%. Analyses were completed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

3.3. Results

Microhardness

The two-way interaction between treatment types and models was significant (p=0.01). The percent change in surface microhardness (%SMHchange) data for both pH-cycling with fluoride intervention and placebo model are shown in Figure 8.

In the pH-cycling with fluoride model there were no statistically significant differences between SDF and SDF+KI in preventing enamel demineralization ($p=0.992$). There were no statistically significant differences between SDF, SDF+KI and KF ($p>0.8$) and they were all more effective in preventing demineralization than $AgNO_3$ and deionized water ($p<0.0001$). There was no difference between $AgNO_3$ and deionized water ($p=0.91$).

In the pH-cycling with placebo model there were no statistically significant differences between SDF and SDF+KI in preventing enamel demineralization ($p=0.4410$). However, KF was more effective in preventing caries lesion formation than SDF and SDF+KI ($p<0.00001$). SDF, SDF+KI and KF were more effective in preventing demineralization than $AgNO_3$ and deionized water ($p<0.0001$). There was no difference between $AgNO_3$ and deionized water ($p=0.6747$).

SDF, SDF+KI, $AgNO_3$ and deionized water treatments resulted in significantly less demineralization with twice-daily fluoride applications than with placebo treatments ($p<0.05$). In the KF group there was no statistically significant difference between pH-cycling with fluoride and placebo models in preventing enamel demineralization ($p=0.510$).

Transverse Microradiography

The two-way interaction between treatment types and models was significant ($p=0.001$). The ΔZ data for both pH-cycling with fluoride and placebo models are shown in Figure 9. In the pH-cycling with fluoride model there was no difference in ΔZ between treatment groups (all $p>0.15$). However, in the pH-cycling with placebo model there were

statistically significant differences in ΔZ between SDF, SDF+KI, and KF compared to $AgNO_3$ and deionized water (all $p<0.01$).

There were statistically significant differences between pH-cycling with fluoride and placebo models for the SDF+KI, $AgNO_3$, and deionized water groups ($p<0.0001$). However, the difference between pH-cycling with fluoride and placebo models was not significant for the SDF and KF groups (both $p>0.05$).

The two-way interaction between treatment types and models was not significant ($p=0.096$); however, both factors were (treatment types – $p=0.002$; models – $p=0.001$). The L data for both pH-cycling with fluoride and placebo models are shown in Figure 10. In both pH-cycling with fluoride model there were statistically significant differences between SDF, SDF+KI, and KF compared to $AgNO_3$ and deionized water ($p<0.0001$). There were statistically significant differences between pH-cycling with fluoride intervention and placebo models ($p=0.001$). No differences in mean lesion mineral distributions between study groups were noted (data not shown).

Color Assessment

The two-way interaction between treatment types and models was significant ($p=0.002$). The ΔL* data for both pH-cycling models and all treatment groups are shown in Figure 11. Irrespective of the pH cycling model and considering only color changes after treatment application, SDF and $AgNO_3$ groups presented significantly lower ΔL* values compared to SDF+KI, KF and deionized water between baseline and post-intervention ($p<0.0001$). Moreover, in the SDF group ΔL* did not significantly change after pH-cycling intervention in both models ($p<0.0001$). In both pH-cycling models, ΔL* values from baseline to post-intervention shown SDF+KI groups had significantly higher ΔL*

values than SDF alone ($p<0.0001$). In the pH-cycling with placebo model, the deionized water group demonstrated statistically significant increase in ΔL* compared to all other groups from baseline to post-intervention ($p<0.0001$).

Twice-daily fluoride application in the pH-cycling with fluoride intervention did not affect the L* values compared to pH cycling with placebo in the SDF, SDF+KI and KF groups ($p\geq0.0535$). In the $AgNO_3$ and deionized water groups, pH-cycling with placebo significantly increased ΔL* values ($p<0.0001$).

3.4. Discussion

SDF has gained growing popularity in treating dentin caries**.** However, there is insufficient evidence about the caries preventive effect of SDF on enamel. To the authors' knowledge**,** no experimental data about the ability of SDF in preventing enamel demineralization under pH-cycling conditions in the presence or absence of twice-daily fluoride application exists. Therefore, this study is innovative because it is the first to examine the specific effect of SDF and SDF+KI as a caries preventative agent on sound enamel.

The chosen pH-cycling model is based on the model by Featherstone et al. (2011) (Featherstone, Stookey et al. 2011) which is a net demineralization model (Featherstone, Stookey et al. 2011). This study mimicked in vivo caries formation in a high-risk patient as a professionally applied intervention was followed by twice-daily at-home application of over-the-counter fluoride toothpaste. This model was able to distinguish between SDF, SDF+KI and KF vs. $AgNO_3$ and deionized water, highlighting longitudinal effects of the fluoride-containing interventions that persisted even after pH cycling. The model was

also sensitive enough to show the effect of twice-daily fluoride application during the pH cycling phase in addition to aforementioned intervention effects.

The present findings suggest that SDF appears to offer an alternative approach in preventing primary coronal caries. Furthermore, KI application after SDF significantly improved the dark staining without affecting the inhibition of demineralization ability of SDF. These findings are highly consistent and strongly support my study hypotheses. Based on the findings of this study, SDF and SDF+KI are more effective in inhibiting demineralization and promoting remineralization than $AgNO_3$ and deionized water (Figure 8). Accordingly, application of KI after SDF did not affect the ability of SDF to prevent demineralization (Figure 8). These results are in agreement with my previous work using a chemical model to induce demineralization on sound enamel (Sorkhdini, Gregory et al. 2020) and a biofilm study employing dentin specimens (Knight, McIntyre et al. 2005).

In both models, SDF, SDF+KI and KF were more effective in their ability to prevent demineralization and promote remineralization than $AgNO_3$ and deionized water (Figure 8). Moreover, there was no difference between SDF, SDF+KI and KF with twice daily fluoride treatments. Nevertheless, KF was superior in preventing demineralization and promoting remineralization than SDF and SDF+KI in pH-cycling with placebo. These results clearly indicate that the caries preventive effect of SDF in this model is a function of the fluoride content and not the silver component or any combination of the two.

Mei et al. mentioned in their review article on dentin that the combination of silver and fluoride in an alkaline solution has a synergistic effect in arresting dentin caries

as silver ions inhibit biofilm growth, whereas fluoride enhances mineral formation, which makes SDF different from other fluoride agents (Mei, Lo et al. 2018). However, the role of silver and fluoride ions on the preventive effect of SDF on sound enamel, is still unclear.

Fluoride, which is the other component of SDF, plays an important role in promoting remineralization and inhibiting enamel demineralization. Fluoride enhances the speed of enamel remineralization and slows down the enamel dissolution process. Fluoride has also been demonstrated to have anti-bacterial activity which may prevent production of acids by bacteria (Mei, Zhao et al. 2016, Hu, Meyer et al. 2018). However, it is still not clear to what extent silver and fluoride ions in SDF display their mechanism of action when applied to enamel (Hu, Meyer et al. 2018).

Based on the VHN results of both pH-cycling models, KF was equally or more effective than SDF. There are several hypotheses I consider as a rationale:

1) Silver ions in SDF (loosely) bound to the enamel surface may act as a barrier for effective remineralization to occur. It is possible that residual silver ions after SDF application or silver ions released from bound silver compounds solubilized during phases of demineralization, which can reprecipitate again, did impair remineralization. This may have reduced the effectiveness of fluoride initially applied as part of the SDF intervention (Zhi, Lo et al. 2013).

2) Secondly, this could be explained by the fact that silver ions seem to precipitate in the pellicle when applied to sound enamel surfaces, which would mean that most of the observed effects are due to the remineralizing action of the higher concentration of fluoride (Li, Liu et al. 2019).

3) Thirdly, fluoride ions from SDF may be hampered from entering the sound enamel since there was no lesion to penetrate. As sound enamel has very low porosity and SDF has high reactivity with the enamel surface, the remineralizing effect of fluoride in SDF may have been decreased (Rosenblatt, Stamford et al. 2009).

4) Lastly, no cariogenic biofilms were included in the pH-cycling models. Thus, the anti-bacterial effect of silver was not considered in the present study. The transverse microradiography data (Figs. 9 and 10) largely mirrored the hardness data, although differences between treatment groups and models were less pronounced. This may be due to the lack of sensitivity of the transverse microradiography technique in precisely evaluating the mineral status of very early, shallow lesions, which agrees with a previous study (Sorkhdini, Gregory et al. 2020).

SDF has been proven to be a relatively safe topical agent from a pharmacokinetics perspective (Vasquez, Zegarra et al. 2012). However, the pronounced and permanent black staining of SDF is a significant aesthetic barrier which greatly impacts its adoption into everyday practice. Parents believe the dark staining on the tooth surface would result in harming psychosocial consequences to their child due to the judgments of other individuals (Roberts, Bradley et al. , Crystal, Kreider et al. 2019). Hence, diminishing the black staining caused by SDF would greatly enhance the opportunity for its universal use (Crystal, Kreider et al. 2019, Garg, Sadr et al. 2019).

In this study, dark staining was observed on the enamel specimens treated with either SDF or $AgNO_3$ (Figure 11). These results were in agreement with studies on dentin (Zhi, Lo et al. 2013) and enamel (Sorkhdini, Gregory et al. 2020). The extent of staining

caused by SDF did not subside during the pH cycling phase, suggesting that the stain is of a tenacious nature and resists repeated acid challenges.

In both pH-cycling models, application of KI after SDF considerably reduced the discoloration caused by SDF (Figure 11). This was in agreement with the outcomes of other studies performed on enamel and dentin (Gupta, Thomas et al. 2019, Zhao, Chu et al. 2019). Moreover, KI was able to permanently prevent staining in enamel caused by SDF-related in both pH-cycling models (Figure 11).

As KF group was strong anti caries treatment the pH-cycling did not affect the color of specimen and did not create white opacity lesion due to demineralization. I noticed an interesting result in both $AgNO_3$ groups in that the staining persisted throughout the pH cycling phase in the fluoride-treated specimens but not in those treated with deionized water (Figure 11). It is likely that the repeated fluoride application resulted in more persistent binding of silver ions, whereas in the absence of fluoride the daily acid challenge lead to continuous dissolution of enamel-bound silver. However, $AgNO_3$ treatment per se did not affect demineralization as demonstrated using a different model previously (Zhi, Lo et al. 2013, Zhao, Mei et al. 2017, Sorkhdini, Gregory et al. 2020). This suggests different reaction products between SDF and enamel vs. $AgNO_3$ and enamel which warrants further exploration. Lastly, in the pH-cycling with placebo model, the deionized water group demonstrated significantly more whiteness than all other groups after pH-cycling intervention because of the formation of white opacity demineralization lesions (Figure 11).

Several limitations must be considered in the interpretation of the present results. The chosen pH cycling period was relatively short, and a longer duration may have been

useful to predict potentially longitudinal effects of SDF in enamel caries prevention. A longer phase would have also allowed to evaluate the impact of a repeat SDF application as conducted clinically. (Crystal, Rabieh et al. 2019). Undoubtedly, pH-cycling models have their own limitations. as they only partially replicate the complex clinical conditions of caries dynamics and natural oral environments (Zhao, Mei et al. 2017). It is inappropriate to compare it directly with the clinical situation. Similarly, it is difficult to compare with the biofilm models used to simulate cariogenic biofilm effects, as these are more complex than a simple, chemical pH-cycling model. The major limitation with regards to chemical pH-cycling is the lack of bacteria and pellicle which are naturally present in the oral environment. For example, acid diffusion is completely altered by the gradient that is caused by an extracellular biofilm matrix, which also controls ion exchange between the enamel surface and the external environment. Ultimately, the pH-cycling model is simplistic.

The results warrant further longitudinal investigation with bacterial pH-cycling models comprising biofilm development to simulate a natural process occurring in the oral cavity to better understand the efficacy of SDF compared to SDF+KI and its individual components.

Under the conditions of this study, SDF appears to be an effective topical agent in the prevention of enamel caries with the mode of action being effective delivery of high concentrations of fluoride ion to the sound enamel surface. While KI helped prevent dark staining caused by SDF, KI did not impair SDF's ability to prevent chemical demineralization. Further clinical research is required to confirm the caries preventive ability of SDF and SDF+KI on enamel.

Figure 7. Schematic of the experimental procedures

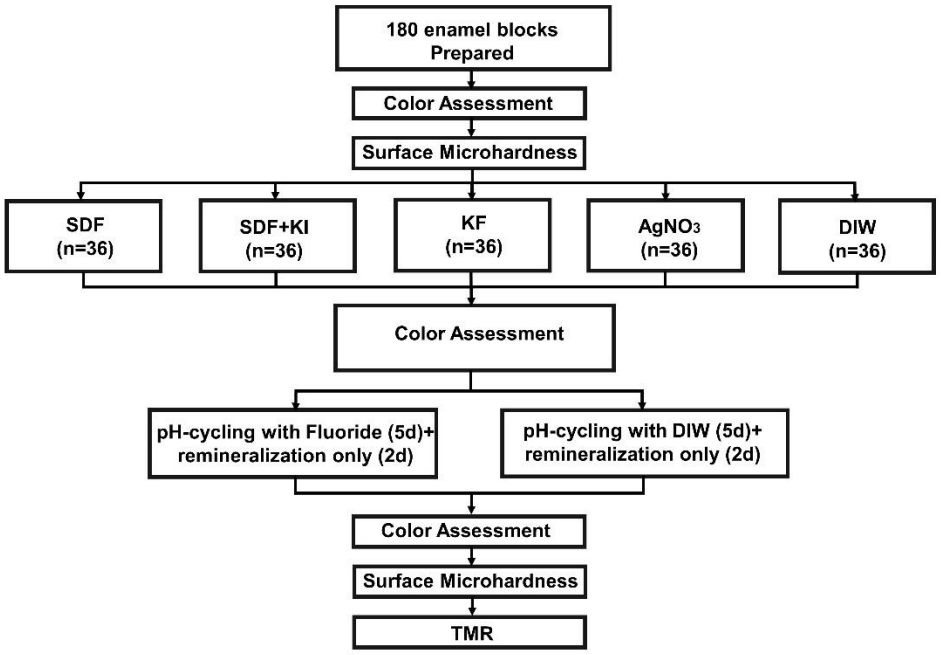

Figure 8. pH cycling with Fluoride Vs. Placebo - percent change in surface microhardness (%SMH change) from baseline

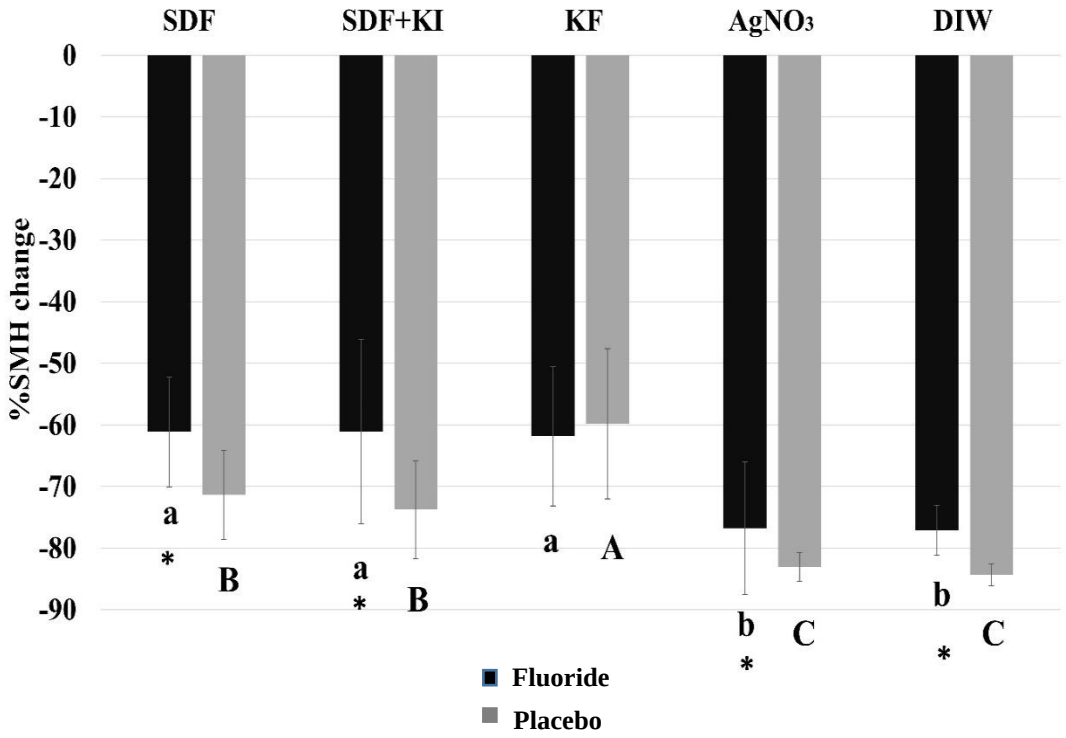

Figure 9. pH cycling with Fluoride Vs. Placebo - change in Integrated Mineral Loss (ΔZ; mean ± standard deviation; all vol%min×µm)

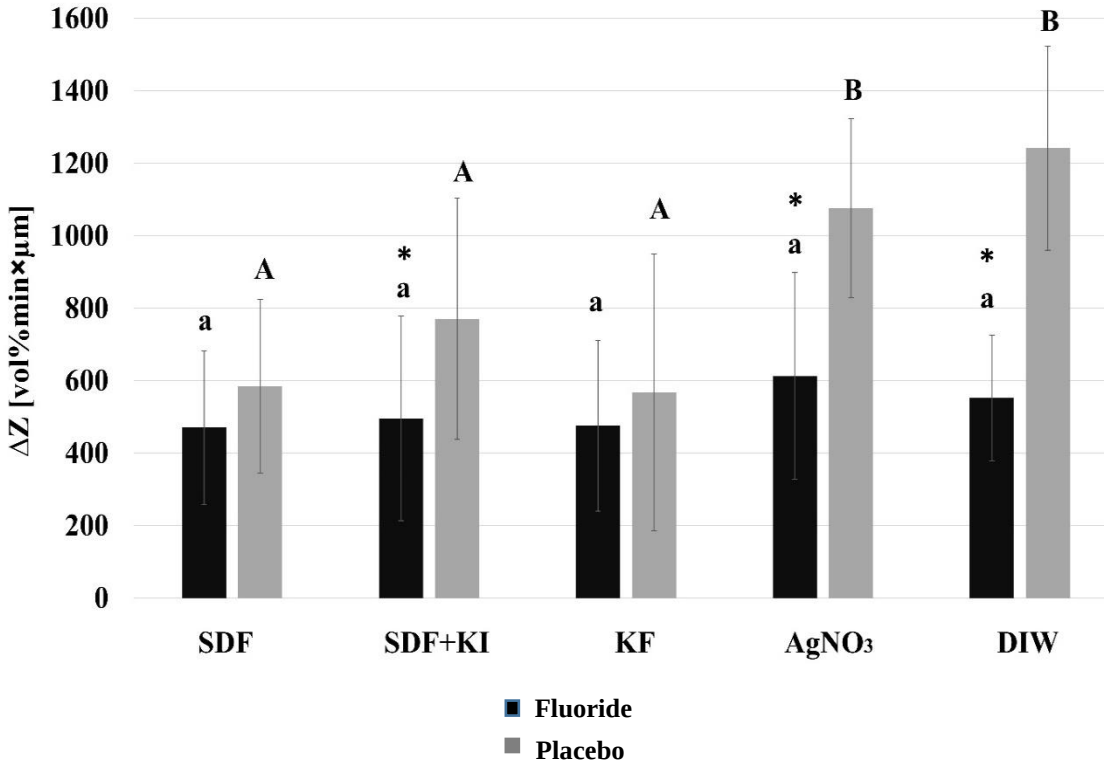

Figure 10. pH cycling with Fluoride Vs. Placebo – change in Lesion Depth (L; mean ± standard deviation; all µm)

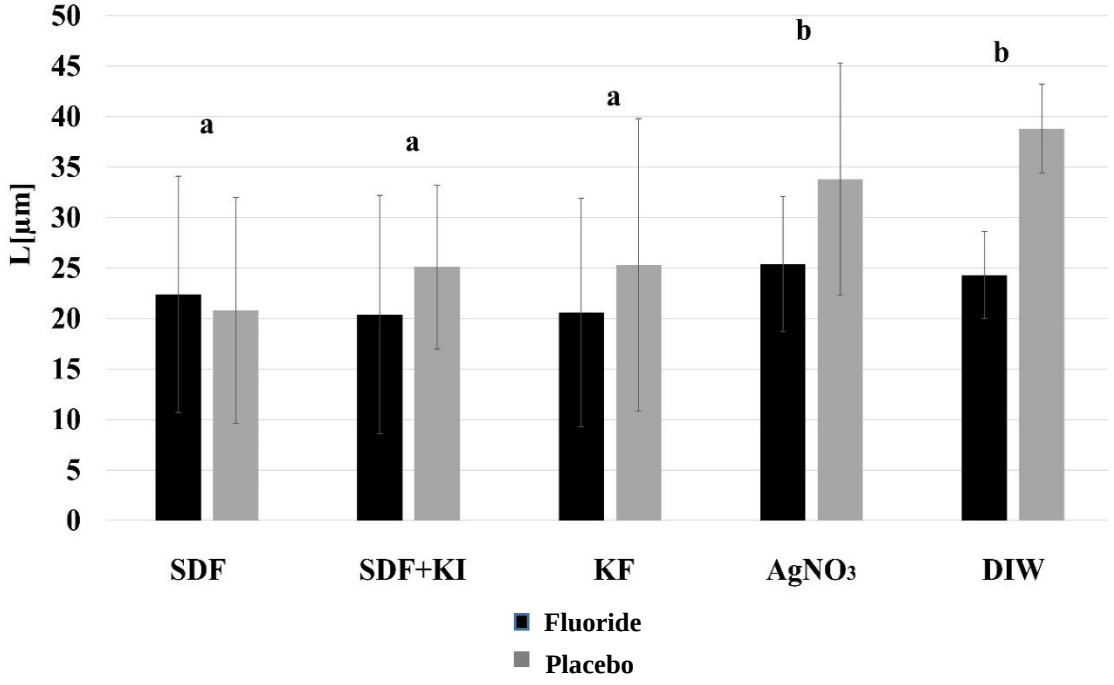

Figure 11. pH cycling with Fluoride Vs. Placebo – color change (ΔL*)

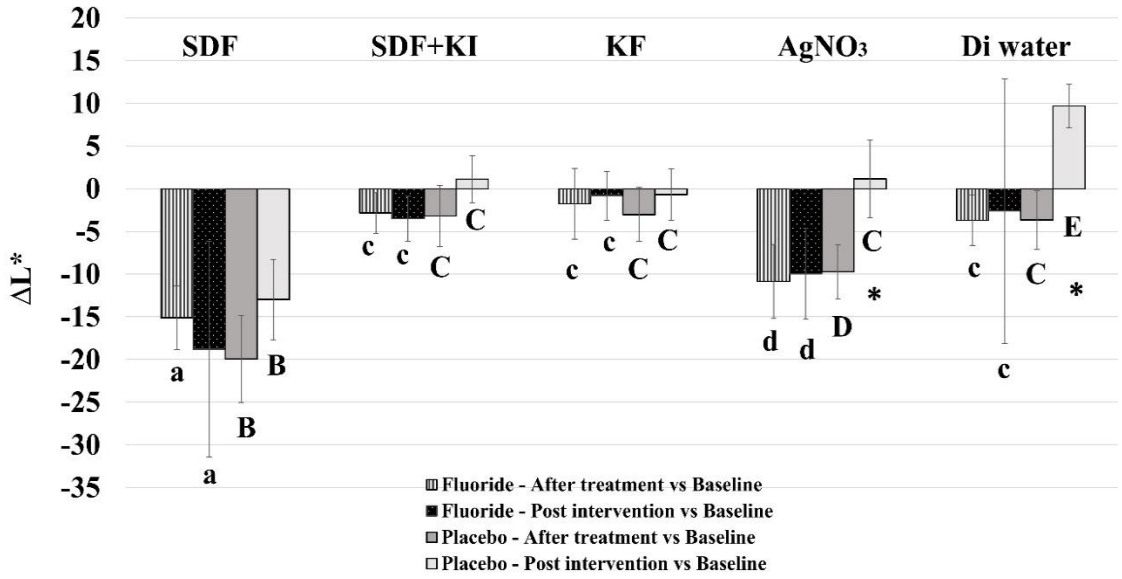

Figure legends

Figure 7. Schematic of the experimental procedures.

Figure 8. %SMHchange data (mean ± standard deviation) as a function of intervention after pH cycling with twice-daily fluoride (black bars) or placebo (gray bars) treatments. Different letters highlight statistically significant differences between treatment groups and within each model. Asterisks highlight statistically significant differences within treatment groups between models.

Figure 9. Integrated mineral loss data (ΔZ; mean ± standard deviation) as a function of intervention after pH cycling with twice-daily fluoride (black bars) or placebo (gray bars) treatments. Different letters highlight statistically significant differences between treatment groups and within each model. Asterisks highlight statistically significant differences within treatment groups between models.

Figure 10. Lesion Depth (L; mean ± standard deviation) data as a function of intervention after pH cycling with twice-daily fluoride (black bars) or placebo (gray bars) treatments. Different letters highlight statistically significant differences between treatment groups.

Figure 11. Color change (ΔL*; mean ± standard deviation) data for both models and all treatment groups. Different letters highlight statistically significant differences between treatment groups and within each model. Asterisks highlight statistically significant differences within treatment groups between models.

CHAPTER 4: THE EFFECT OF SILVER DIAMINE FLUORIDE IN REMINERALIZATION OF EARLY ENAMEL CARIES LESIONS

4.1. Introduction

Untreated dental caries lesions in children can impact their quality of life and lead to problems with food intake, growth, difficulty sleeping, self-esteem, and performance of daily living activities (Ferraz, Nogueira et al. 2014, Ramos-Jorge, Pordeus et al. 2014). Surgical restorative treatment is the current, traditional approach, which focuses on restoring the damage from caries. This requires complicated equipment, highly trained dental health personnel, and an expensive procedure. These factors may influence and restrict access, especially in medically underserved individuals and those who are unable to tolerate complicated treatment (Seifo, Robertson et al. 2020). Moreover, with the current medical, emotional and financial crisis surrounding the latest coronavirus (COVID-19) outbreak, there may be a greater need for silver diamine fluoride (SDF) in dental clinics as a quick, inexpensive and relatively safe solution to treat dental caries.

Over the past few years, remineralization of dental caries has gained recognition as a minimally invasive approach to dental caries treatment (Zhi, Lo et al. 2012, Dorri, Martinez-Zapata et al. 2017, Gao, Zhao et al. 2018). However, current methods of early caries management do not appear to promote remineralization effectively. This suggests the need for innovative and alternative approaches for the management of early dental caries that are minimally invasive and less traumatic for children (Selwitz, Ismail et al. 2007).

Silver diamine fluoride (SDF; $AgF(NH_3)_2$) is a promising intervention that may enhance the remineralization of initial caries lesions. SDF has the advantages of inducing

only minimum discomfort, requiring minimal training, and it is cost-effective and should therefore be affordable to most communities (Mei, Lo, and Chu 2016). Systematic reviews concluded that the application of SDF can be an effective therapeutic option for caries management at the subclinical level among preschool children, particularly those with poor access to dental care (Gao et al. 2016) (Duangthip et al. 2018). The higher effectiveness of SDF could be attributed to high concentrations of silver (25.5%) and fluoride (44,880 ppm) which may lead to greater efficacy in management of dental caries compared to fluoride varnish (Sayed, Matsui et al. 2018).

Although SDF has been used to arrest dentine caries in young children, its role in rehardening active subclinical caries lesions in enamel has not been determined yet (Chu, Lo et al. 2002, Llodra, Rodriguez et al. 2005, Gao, Zhao et al. 2016). Understanding the mechanism behind SDF remineralization promotion could reduce the knowledge gap and advance scientific evidence of its clinical success (Mei, Lo, and Chu 2018). Furthermore, SDF leaves black staining on the treated tooth surface which may not be pleasing to the patient. Thus, SDF is not widely used on the visible tooth surfaces (Punyanirun et al. 2018). To mitigate staining, Knight et al. suggested using potassium iodine (KI), which reacts with the residual silver ions to eliminate the color stain effect (Knight, McIntyre et al. 2006). There is a disagreement in the literature about whether KI is effective in preventing dark staining without impacting the remineralization promotion of early caries lesions in enamel.

Lastly, to the best of our knowledge, there is no solid data about the potential SDF-protein interactions and how these interactions affect the remineralization process in enamel.

Accordingly, remineralization models with and without mucin were used in this study to evaluate, 1) the remineralization promotion efficacy of SDF for caries treatment in enamel, 2) if applying KI after SDF affects the remineralization promotion efficacy of SDF while simultaneously retarding staining issues, and 3) potential SDF-protein interactions and how these interactions affect the remineralization process. We hypothesized that, a) SDF is an effective agent in promoting remineralization, b) applying KI after SDF application can lessen dark staining while not adversely affecting the remineralization promotion efficacy of SDF and, c) mucin in artificial saliva (AS) will enhance the ability of SDF to promote remineralization of early caries lesion.

4.2. Materials and Methods

Study design

The study was exempted from IRB overview IRB #: NS0911-07. The experimental procedure is depicted in Figure 12. One hundred and eighty polished human permanent enamel specimens were used in this study. After baseline color and surface microhardness assessments, artificial caries lesions were created by 36 h immersion into a demineralizing solution. After color and surface microhardness assessments, specimens were divided into five treatment groups (SDF, SDF+KI, $AgNO_3$, KF and DIW). Color assessment was performed following the treatment application. Specimens were then randomized into two 4-day, continuous remineralization models: 1) remineralization with mucin or 2) remineralization without mucin. Finally, all specimens were evaluated for changes in color and surface microhardness.

Specimen selection and preparation

One hundred and eighty sound extracted human permanent teeth (buccal and/or lingual surfaces of predominantly molars and premolars from dental clinics' anonymous donations) with no defects were used in this study. Each tooth was cut into 4×4 mm specimens using a low-speed saw (Iso Met, Buehler, Lake Bluff, IL, USA) as described previously (Sorkhdini, Gregory et al. 2020). The thickness of the final specimens was 1.7–2.2 mm. Specimens were evaluated under a Nikon SMZ 1500 stereomicroscope at 20× magnification for cracks, hypomineralized areas or other flaws in the enamel surface that would disqualify them from being used in the study. Specimens were stored at 100% relative humidity at 4° C until further use.

Sound enamel color assessment

Color assessments were performed to assess color changes among different treatments as described previously (Alshara, Lippert et al. 2014). Commision Internationale de l'Eclairage (CIE) L* values were recorded (L^*_{sound}).

Sound enamel surface microhardness

Sound enamel samples were evaluated for SMH using a microhardness tester as described previously (2100 HT; Wilson Instruments, Norwood, MA, USA) (Sorkhdini, Gregory et al. 2020). SMHsound was determined from the respective indentation lengths and measured using dedicated image analysis software (Clemex CMT HD version 6.0.011, Clemex Technologies Inc., Longueuil, Quebec, Canada). Only enamel specimens that met the criteria of $300 \leq SMHsound \leq 400$ were utilized.

Specimen stratification

One hundred and eighty enamel specimens were stratified into five treatment groups with 36 specimens per treatment group to ensure that there were no significant differences in SMHsound between groups.

Artificial caries lesion creation

Artificial lesions were formed in the enamel specimens by a 36 h-immersion into a solution of 0.1 M lactic acid, 0.2% Carbopol 907, 3.0 mM $CaCl_2$ x $2H_2O$, 6.0 mM KH_2PO_4, 63.0 mM KCl, and 3.1 mM NaN_3, with the pH adjusted to 5.0 at 37ºC (Lippert 2017). After lesion creation, specimens were rinsed with deionized water, and stored at approx. 100 % relative humidity at 4°C.

Post-demineralization assessment

Post- demineralization color assessment

After inducing caries lesions color assessments were performed on all specimens as described above. L* was measured for each specimen (L^*_{lesion}).

Surface microhardness change

All specimens were again subjected to surface microhardness measurements as described above, and changes vs. baseline was calculated. A second set of four indentations was placed on each specimen in close proximity of the SMHsound, indentations yielding SMHpostdemin.

Interventions

The studied interventions were: SDF, SDF+KI, $AgNO_3$, KF and DIW (placebo).

- SDF: 38% SDF (Advantage Arrest, Elevate Oral Care LLC, Florida, USA) solution; nominally 253,900 ppm Ag; 44,800 ppm F (Advantage Arrest, Fl, USA)

- SDF+KI: SDF application followed by supersaturated KI application (Potassium iodide 39% w/v solution, 30315, Sigma–Aldrich, St. Louis, US)
- KF: fluoride control; 44,800 ppm F (Potassium fluoride 60238, Sigma–Aldrich, St. Louis, USA)
- $AgNO_3$: silver control; 253,900 ppm Ag (Silver nitrate 31630, Sigma–Aldrich, St. Louis, USA)
- DIW: negative control

To apply SDF solution a micro applicator (Regular; Premium Plus International Ltd., Hong Kong, China) was used. All other solutions (KI, KF, $AgNO_3$ and DIW) were applied to the specimens with a micro-brush (Premium Plus Regular Tip Micro A microbrush).

A saturated KI solution was applied immediately after SDF application for the SDF+KI group, until the creamy yellow solution turned clear (Zhao, Mei et al. 2017). Five minutes later all treatments were rinsed with DIW.

Post-treatment color assessment

Immediately after interventions were applied, color assessments were performed again as described above**.** L* was measured for each specimen ($L^*_{intervention}$) and the following variable was calculated: $\Delta L^*_{intervention} = L^*_{intervention} - L^*_{lesion}$.

Treatment Regime

Immediately following color assessment, all specimens were remineralized for 4 days at room temperature, under constant agitation (150 rpm) and using artificial saliva with or without mucin (2.20 g/L; gastric mucin[American Laboratories Inc., NE, USA]). Artificial saliva had the following composition: 1.5 mM $CaCl_2 \times 2\ H_2O$, 0.9 mM

KH_2PO_4, 130 mM KCl, 20 mM HEPES, pH 7.0 (Vieira, Bayram et al. 2017). Saliva solutions were replaced every 24 h (Scaramucci, Borges et al. 2015). To avoid cross-contamination, specimens from each of the ten subgroups were remineralized separately.

Post-remineralization assessments

Color assessment

Color assessments were performed on all specimens after remineralization as described above. L* was measured for each specimen (L^*_{remin}) and the following variable was calculated: $\Delta L^*_{remin} = L^*_{remin} - L^*_{intervention}$.

Surface microhardness change

Surface microhardness measurements were performed again, as described above. A third set of four indentations was placed on each sample in close proximity to the previous set, yielding VHNpostremin. The extent of percent change in SMH for each individual specimen was calculated as follows: ΔVHN = VHNpostremin – VHNpostdemin.

Statistical considerations

Sample size calculation

With a sample size of 18 specimens per group in each intervention, the study had 80% power to detect a difference of 10% for ΔVHN, and 15% for color changes (ΔL*). The calculations assumed two-sided tests conducted at a 5% significance level for each type of comparison, with coefficients of variance estimated at 0.1 for ΔVHN and 0.15 for ΔL*.

Statistical Analysis

Two-way ANOVA was utilized to calculate ΔVHN and ΔL* to observe the impact of treatment types and models, as well as interactions between treatment types and models.

All pair-wise comparisons from ANOVA analysis were conducted using Fisher's Protected Least Significant Differences to control the overall significance level at 5%. Analyses were assessed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

4.3. Results

Microhardness

There were no statistically significant differences in the extent of surface softening between treatment groups after artificial lesion creation (p=0.3060).

The two-way interaction between different treatment options and models was significant (p<0.0001). The ΔVHN values for both remineralization models are shown in Figure 13.

ΔVHN

In the mucin model, there were statistically significant differences between SDF and SDF+KI in promoting enamel remineralization (p=0.033). Also, there were statistically significant differences between SDF and KF (p=0.0071) and both SDF+KI and KF were more effective in promoting remineralization than SDF. SDF, SDF+KI, and KF were significantly more effective in promoting enamel remineralization than $AgNO_3$ and DIW (p<0.0001). There was no difference between $AgNO_3$ and DIW (p=0.23).

In the remineralization without mucin model, there were statistically significant differences between SDF and SDF+KI, and KF in promoting enamel remineralization (p<0.0001). SDF+KI, and KF were both more effective in promoting remineralization than SDF (p<0.0001). There was no difference between SDF and DIW (p=0.11). SDF+KI and KF were both significantly more effective in promoting enamel remineralization than SDF, $AgNO_3$ and DIW (p<0.0001). Also, there were no statistically

significant differences between SDF+KI and KF ($p=0.74$) and between $AgNO_3$ and DIW ($p=0.24$).

Only SDF resulted in significantly more remineralization in the presence vs. the absence of mucin in artificial saliva ($p<0.0001$), while there were no statistically significant differences in the other groups ($p\geq0.0824$).

Color Assessment

There were no statistically significant differences in L* between treatment groups after artificial lesion creation ($p=0.6506$). The two-way interaction between different treatment options and models was significant ($p=0.0037$). The ΔL* data for both models and all treatment groups are shown in Figure 14.

Irrespective of the remineralization model and considering only color changes after treatment application, SDF and $AgNO_3$ groups presented significantly lower ΔL* values compared to SDF+KI, KF and DIW between baseline and post-remineralization ($p<0.0001$). Moreover, in the SDF group ΔL* did not significantly change after remineralization in both models ($p<0.0001$). In both remineralization models, ΔL* values from baseline to post-remineralization demonstrated that SDF+KI groups had significantly higher ΔL* values than SDF alone ($p<0.0001$).

In the remineralization with mucin model, the SDF+KI group demonstrated a statistically significant decrease in ΔL* values compared to KF and DIW group from baseline to post- remineralization ($p<0.0001$). Adding mucin to the remineralization model did not affect the ΔL* values compared to remineralization without mucin in the SDF, KF, $AgNO_3$, and DIW groups ($p\geq0.11$).

4.4. Discussion

SDF has been shown to be a valuable agent for the management of active dentin caries lesions. However, there is a lack of evidence about the remineralization promotion efficacy of SDF and SDF+KI on early enamel caries lesions**.** To our knowledge, this was the first study to examine the remineralizing efficacy of SDF and SDF+KI on early enamel caries lesions.

The present study mimicked the short-term remineralizing effect of SDF when applied to subclinical enamel caries lesions. Remineralization models using AS with and without mucin were used to investigate potential silver-salivary protein interactions, as SDF has been shown to react and interact with dentin collagen (Zhao, Gao et al. 2018). Mucin was chosen because it accounts for a large percentage (7–26%) of total salivary protein (Levine 1993, Slomiany, Murty et al. 1996). Mucin has been shown to inhibit demineralization against erosive attacks on enamel (Nieuw Amerongen, Oderkerk et al. 1987) and promote remineralizing characteristics of an artificial saliva by increasing calcium diffusion into the initial lesions (Kielbassa, Shohadai et al. 2001, Meyer-Lueckel, Umland et al. 2004). However, the role of mucin in remineralization of early enamel caries lesions and possible mucin interactions with SDF, silver and fluoride are still unknown.

The present models were able to distinguish the remineralization efficacy between fluoride and non-fluoride interventions, thereby highlighting its potential clinical relevance.

Based on the results of this study, SDF is a comparatively ineffective treatment in the short term remineralization of early enamel caries lesions. Moreover, KI application

after SDF significantly lessened the dark staining associated with SDF, and at the same time promoted the remineralization ability of SDF. These findings do not support this study's hypotheses.

The present data demonstrates that the co-application of high concentrations of fluoride and silver ions do not necessarily enhance remineralization. The effectiveness and the role that fluoride and silver ions in SDF have in promoting remineralization of enamel is still unknown. It has been suggested that the main mechanism behind the remineralizing ability of SDF is because of the effect of the fluoride ions. Accordingly, it has been proposed that SDF, by enhancing the fluoride content in enamel, inhibits dissolution of tooth mineral by acid via absorption onto the enamel crystals. This is followed by stimulating the remineralization of the enamel crystals. This mechanism leads to the eventual formation of a fluorapatite-like (FAP) layer and a reduction of hydroxyapatite solubility which increases resistance to a subsequent acid attack. It was also shown to perturb bacterial metabolism by inhibiting bacterial enzymes (Featherstone 1999, Stoodley, Wefel et al. 2008).

The silver ion is believed to have a substantial role in the antibacterial and anticaries properties of SDF. Nevertheless, the remineralization ability of silver on early enamel caries lesions has not been studied yet. Based on the results for SDF and $AgNO_3$, silver ions are not effective in promoting remineralization of enamel caries lesions. This suggests different reaction products between SDF and enamel vs. $AgNO_3$ and enamel, which warrants further exploration. It is worth mentioning that the management of caries with $AgNO_3$ must be followed by fluoride varnish application, which acts as a protective layer to prevent $AgNO_3$ from being washed away by saliva (Zhao, Mei et al. 2017).

These observations are somewhat different from two previous studies (Kielbassa, Shohadai et al. 2001, Meyer-Lueckel, Umland et al. 2004) which demonstrated remineralization promotion in the presence of mucin due to the possible interaction between mucin, calcium and fluoride. Our findings in the SDF group suggest an interplay between mucin, silver and fluoride which was beyond the scope of this study and warrants further investigation.

Since SDF is a valuable agent for caries prevention and treatment, understanding the mechanism behind the remineralization promotion of SDF provides a better insight into practice innovation (Zhi, Lo et al. 2013). Based on the VHN results of both remineralization models (Figure 13), KF was more effective than SDF in promoting remineralization. We propose several hypotheses as a rationale:

1) SDF arrested the lesions and therefore not only slowed down further demineralization but also remineralization. Accordingly, if a longer remineralization challenge was conducted the difference between SDF and $AgNO_3$ would likely not be significant, as SDF releases fluoride over time which promotes remineralization.

2) Silver ions from SDF may (loosely) coat the enamel lesions and act as a barrier for efficient remineralization to take place, which may reduce the effectiveness of fluoride ions as another component of the SDF solution from entering the lesions. It may also decrease the stimulating effect of fluoride ions in remineralization (Zhi, Lo et al. 2013).

Moreover, based on the VHN results of both remineralization models, KF was as effective as SDF+KI in promoting remineralization. The reason for this is possibly that, although extra silver ions are removed by KI, the effect of silver ions on remineralization

is likely to be insignificant (Figure 13). This agrees with our previous studies, which showed that silver ions do not appear to interact in de- and remineralization processes (Sorkhdini, Crystal et al. 2020, Sorkhdini, Gregory et al. 2020).

Patient satisfaction is an important indicator for the quality of health care. For young children, parents' satisfaction may play a major role in affecting treatment adherence and success of caries intervention (Duangthip et al. 2018). SDF has a major adverse effect in that it stains the tooth surface black. In the presence of oxygen, Ag_3PO_4, AgO_2 and AgS_2 compounds form on the tooth surface after SDF application. Consequently, these byproducts turn Ag+ to metallic silver nanoparticles which after light exposure causes the tooth tissue to stain (Crystal and Niederman 2019, Li, Liu et al. 2019, Zhao, Chu et al. 2019). In this study, dark staining was noted on the demineralized enamel samples treated with either SDF or $AgNO_3$, before and after remineralization (Figure 14). These results were in agreement with studies on sound enamel (Sorkhdini, Gregory et al. 2020). The extent of staining caused by SDF did not lessen during the short remineralization challenge, indicating that the stain is of a persistent nature and resists the remineralization process.

In both remineralization models, application of KI after SDF noticeably diminished the discoloration caused by SDF (Figure 14). This was in agreement with the outcomes of other studies performed on sound enamel and dentin (Gupta, Thomas et al. 2019, Zhao, Chu et al. 2019, Sorkhdini, Crystal et al. 2020, Sorkhdini, Gregory et al. 2020). However, these are the results from short-term in vitro studies. Clinical data suggests, however, that KI is ineffective in mitigating SDF staining (Li, Lo et al. 2016). A potential explanation for this discrepancy may lie in the presently observed protein,

SDF and KI interplay. In our study, the ability of KI to lessen the extent of staining caused by SDF was negatively affected by mucin (Figure 14). It can be speculated that silver was retained more strongly in the lesions in the presence of mucin, or that more silver was retained due to the co-presence of mucin, thereby limiting the efficacy of KI.

The following study limitations must be considered when interpreting the present findings. This laboratory study did not include demineralization periods as we aimed to isolate the remineralizing ability of SDF. As de- and remineralization phases occur in the oral cavity, chemical and/or bacterial pH-cycling models could be helpful in understanding the remineralization promotion and protein interaction of SDF and SDF+KI under in vivo-like conditions. Only the immediate remineralizing effect of SDF was assessed presently but not its ability to promote remineralization long-term. Possibly applying SDF for a longer period, or a repeated application of SDF, may be more effective in promoting remineralization of early caries lesions. We initially considered including a human saliva group in this study. However, due to COVID-19 we were not able to. Using human saliva would have undoubtedly allowed us to better understand SDF-protein interactions.

Conclusion

Considering the limitations of this in vitro study, SDF was ineffective in remineralizing early enamel caries lesions. The co-presence of mucin during remineralization enhanced the efficacy of SDF which warrants further investigation. KI helped prevent dark staining caused by SDF. SDF+KI appears to be an effective topical agent in the short-term remineralization promotion of enamel caries lesions. Further clinical research on SDF is needed before it can be implemented more widely in primary caries prevention.

Figure 12. Schematic of the experimental procedures

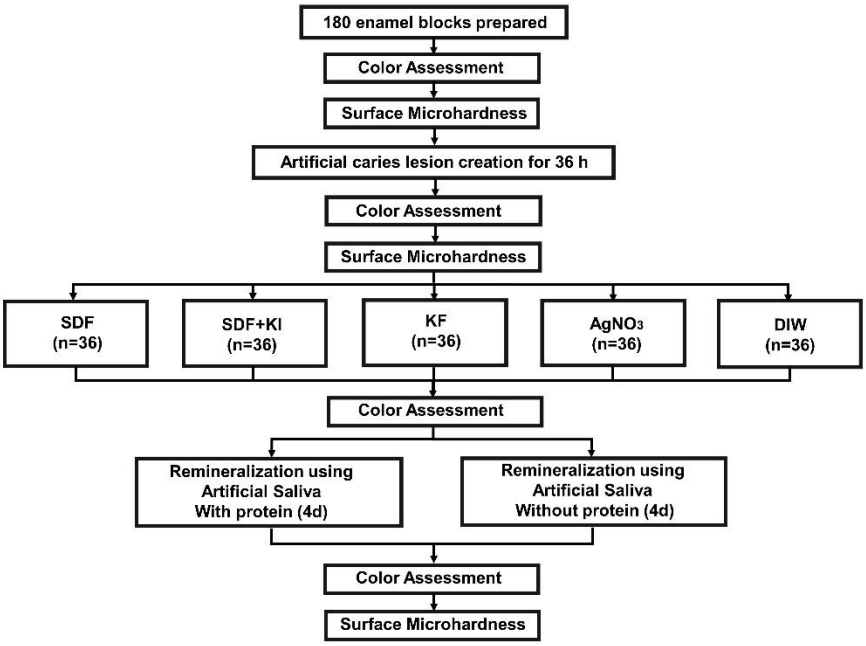

Figure 13. Remineralization with and without protein–change in surface microhardness (ΔVHN) post remineralization vs post demineralization

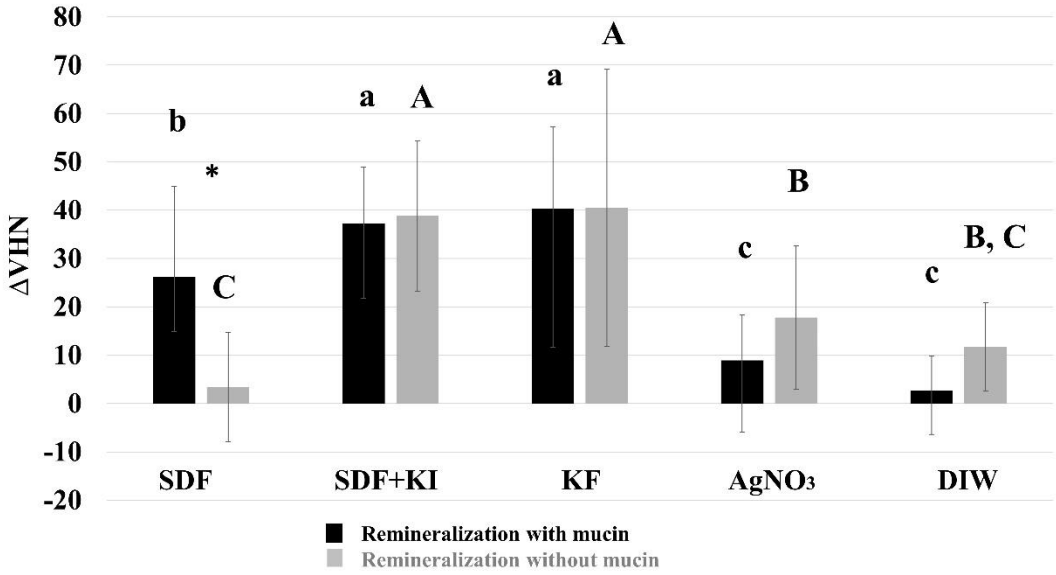

Figure 14. Remineralization with and without mucin – color change (ΔL*)

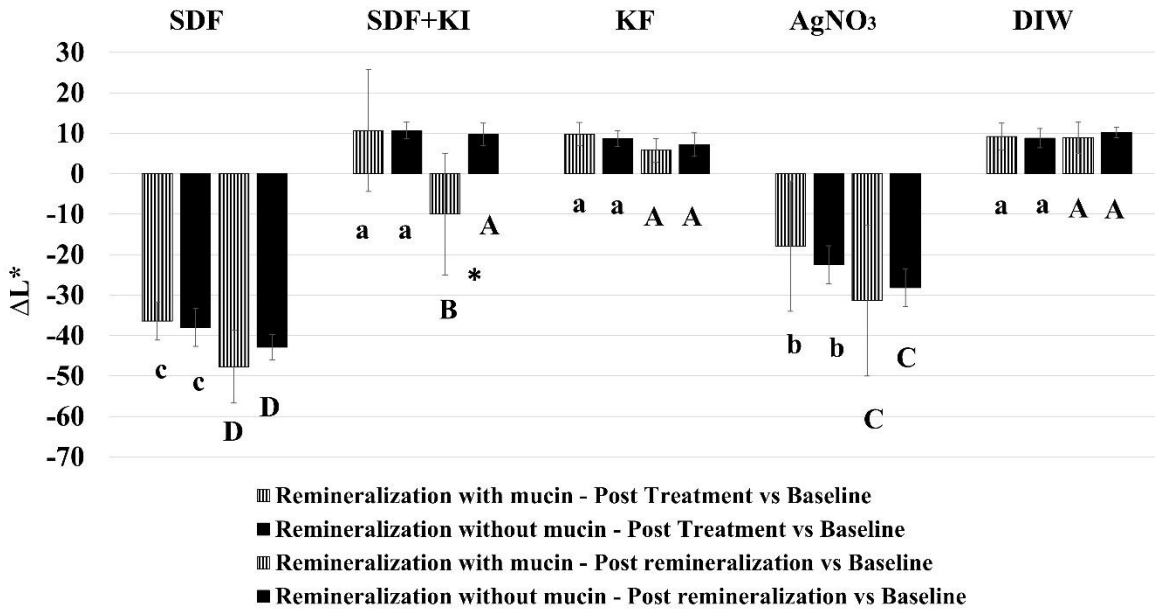

Figure legends

Figure 12. Flowchart of the experimental procedures.

Figure 13. ΔVHN data (mean ± standard deviation) as a function of intervention after remineralization with mucin (black bars) or remineralization without mucin (gray bars) treatments. Different letters highlight statistically significant differences between treatment groups and within each model. Asterisks highlight statistically significant differences within treatment groups between models.

Figure 14. Color change (ΔL*; mean ± standard deviation) data for both models and all treatment groups. Different letters highlight statistically significant differences between treatment groups and within each model. Asterisks highlight statistically significant differences within treatment groups between models.

CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

SDF has gained growing popularity in treating dental caries. This project provided high-quality evidence for using SDF in managing enamel caries compared to SDF+KI, $AgNO_3$, KF and DIW. Moreover, this study provided a safe treatment option for arrestment of secondary enamel caries and the prevention of primary enamel caries to patients with aesthetic concerns regarding SDF.

In Chapter 2, I explored the effectiveness of SDF, SDF+KI, $AgNO_3$, KF, and DIW in preventing enamel demineralization using chemical and biofilm models.

In Chapter 3, I explored the effectiveness of SDF, SDF+KI, $AgNO_3$, KF, and DIW in preventing enamel demineralization using the pH-cycling model as described by Featherstone et al. (2011) (Featherstone, Stookey et al. 2011) with 275 ppm fluoride (mimicking a conventional fluoride dentifrice after dilution during brushing) and deionized water as a placebo.

In Chapter 4, I explored the efficacy of SDF, SDF+KI, $AgNO_3$, KF, and DIW on the remineralization of active early enamel caries lesions in the presence or absence of mucin, a protein found in saliva.

Based on these studies, I conclude the following:

Chapter 2: A distinct difference in the comparative efficacy of SDF vs. SDF+KI was noted between the chemical and biofilm models. While both were equally and more effective than all other interventions in preventing enamel demineralization in the chemical model, this was not the case in the biofilm model. Here, KI impaired the efficacy of SDF. There are several possible explanations for the present observations:

1. KI may reduce silver ion bioavailability, thus the silver ions are not able to bind to and kill bacteria (anti-bacterial effect of silver).
2. KI increases the organic acid production of bacteria, which in turn causes increased demineralization of tooth structures.
3. The combination of SDF+KI may promote bacterial enzymes involved in carbohydrate metabolism and sugar uptake, thus leading to increased demineralization.

In both models, SDF and SDF+KI were superior in their ability to prevent caries lesion formation than $AgNO_3$ and DIW. SDF was more effective than KF in both biofilm and chemical models; however, this difference was not significant in the biofilm model. This discrepancy between models can be due to a host of reasons including the interaction of the biofilm with the enamel surface, different degrees of attachment of the biofilm, biofilm growth and acid production. Based on the results of the study, SDF may offer an alternative biological approach in preventing primary coronal caries in the future. KI application after SDF significantly improved the dark staining and helped enhance the aesthetic outcome by stain reduction.

Chapter 3: This model was able to distinguish between SDF, SDF+KI and KF vs. $AgNO_3$ and DIW, highlighting longitudinal effects of the fluoride-containing interventions that persisted even after pH cycling. The model was also sensitive enough to show the effect of twice-daily fluoride application during the pH cycling phase in addition to the aforementioned intervention effects.

The present findings suggest that SDF appears to offer an alternative approach in preventing primary coronal caries. Furthermore, KI application after SDF significantly

reduced the dark staining without affecting the caries-arresting effect of SDF. These findings are highly consistent and strongly support the study hypotheses. These results are in agreement with my previous work using a chemical model to induce demineralization on sound enamel (Sorkhdini, Gregory et al. 2020) and a biofilm study employing dentin specimens (Knight, McIntyre et al. 2005). In both models, SDF, SDF+KI and KF were more effective in their ability to prevent demineralization and promote remineralization than $AgNO_3$ and DIW. Moreover, there was no difference between SDF, SDF+KI and KF with twice daily fluoride treatments. Nevertheless, KF was superior in preventing demineralization and promoting remineralization to SDF and SDF+KI in pH-cycling with placebo. These results clearly indicate that the caries preventive effect of SDF in this model is a function of the fluoride content and not the silver component or any combination of the two.

Chapter 4: This study mimicked the short-term remineralizing effect of SDF when applied to subclinical enamel caries lesions. Remineralization models using AS with and without mucin were used to investigate potential silver-salivary protein interactions, as SDF has been shown to react and interact with dentin collagen (Zhao, Gao et al. 2018).

This model was able to distinguish the remineralization efficacy between fluoride and non-fluoride interventions, thereby highlighting its potential clinical relevance. Based on the results of this study, SDF is a comparatively ineffective treatment in the short term remineralization of early enamel caries lesions. Moreover, KI application after SDF significantly lessened the dark staining associated with SDF, and at the same time promoted the remineralization ability of SDF. The co-presence of mucin during remineralization enhanced the efficacy of SDF which warrants further investigation.

These findings do not support this study's hypotheses. These results differ from our previous work using a biofilm model to induce demineralization on sound enamel, in which KI application significantly diminished the anti-caries efficacy of SDF (Sorkhdini et al., 2020).

The present data demonstrates that the co-application of high concentrations of fluoride and silver ions do not necessarily enhance remineralization.

Recommendations for further research

Based on the findings from this dissertation, the following recommendations were developed:

- Longitudinal studies on the anti-caries efficacy of SDF and its ability to promote remineralization, and inhibition of demineralization
- Studies on the effects of SDF and SDF+KI under bacterial pH-cycling models should be conducted to better understand the efficacy of SDF compared to SDF+KI and its individual components.
- Analyze lactic acid production in future biofilm studies to verify whether applying KI can cause an increase in acid production or not
- Finally, further clinical research to study the caries preventive ability of SDF and SDF+KI on enamel in biannual applications.

REFERENCES

Alshara, S., F. Lippert, G. J. Eckert and A. T. Hara (2014). "Effectiveness and mode of action of whitening dentifrices on enamel extrinsic stains." Clin Oral Investig **18**(2): 563-569.

Ayoub, H., R. Gregory, Q. Tang and F. Lippert (2019). "The influence of salivary conditioning on biofilm-mediated enamel demineralization(in press)." J. Appl. Oral Sci.

Blackburn, J., M. A. Morrisey and B. Sen (2017). "Outcomes Associated With Early Preventive Dental Care Among Medicaid-Enrolled Children in Alabama." JAMA Pediatr **171**(4): 335-341.

Buzalaf, M. A., A. R. Hannas, A. C. Magalhaes, D. Rios, H. M. Honorio and A. C. Delbem (2010). "pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations." J Appl Oral Sci **18**(4): 316-334.

Byeon, S. M., M. H. Lee and T. S. Bae (2016). "The effect of different fluoride application methods on the remineralization of initial carious lesions." Restor Dent Endod **41**(2): 121-129.

Chu, C. H., E. C. Lo and H. C. Lin (2002). "Effectiveness of silver diamine fluoride and sodium fluoride varnish in arresting dentin caries in Chinese pre-school children." J Dent Res **81**(11): 767-770.

Crystal, Y. O., M. N. Janal, S. Yim and T. Nelson (2020). "Teaching and utllization of SDF and Hall-style crowns in US pediatric dentistry residency programs." J Am Dent Assoc **in press**.

Crystal, Y. O., B. Kreider and V. H. Raveis (2019). "Parental Expressed Concerns about Silver Diamine Fluoride (SDF) Treatment." J Clin Pediatr Dent **43**(3): 155-160.

Crystal, Y. O., A. A. Marghalani, S. D. Ureles, J. T. Wright, R. Sulyanto, K. Divaris, M. Fontana and L. Graham (2017). "Use of Silver Diamine Fluoride for Dental Caries Management in Children and Adolescents, Including Those with Special Health Care Needs." Pediatr Dent **39**(5): 135-145.

Crystal, Y. O. and R. Niederman (2016). "Silver Diamine Fluoride Treatment Considerations in Children's Caries Management." Pediatr Dent **38**(7): 466-471.

Crystal, Y. O. and R. Niederman (2019). "Evidence-Based Dentistry Update on Silver Diamine Fluoride." Dent Clin North Am **63**(1): 45-68.

Crystal, Y. O., S. Rabieh, M. N. Janal, S. Rasamimari and T. G. Bromage (2019). "Silver and fluoride content and short-term stability of 38% silver diamine fluoride." J Am Dent Assoc **150**(2): 140-146.

De Menezes Oliveira, M. A., C. P. Torres, J. M. Gomes-Silva, M. A. Chinelatti, F. C. De Menezes, R. G. Palma-Dibb and M. C. Borsatto (2010). "Microstructure and mineral composition of dental enamel of permanent and deciduous teeth." Microsc Res Tech **73**(5): 572-577.

Delbem, A. C., M. Bergamaschi, K. T. Sassaki and R. F. Cunha (2006). "Effect of fluoridated varnish and silver diamine fluoride solution on enamel demineralization: pH-cycling study." J Appl Oral Sci **14**(2): 88-92.

Dorri, M., M. J. Martinez-Zapata, T. Walsh, V. C. Marinho, A. Sheiham Deceased and C. Zaror (2017). "Atraumatic restorative treatment versus conventional

restorative treatment for managing dental caries." The Cochrane database of systematic reviews **12**(12): CD008072-CD008072.

Dye, B. A., G. Thornton-Evans, X. Li and T. J. Iafolla (2015). "Dental caries and sealant prevalence in children and adolescents in the United States, 2011-2012." NCHS Data Brief(191): 1-8.

Featherstone, J. D. (1999). "Prevention and reversal of dental caries: role of low level fluoride." Community Dent Oral Epidemiol **27**(1): 31-40.

Featherstone, J. D. and S. Doméjean (2012). "The role of remineralizing and anticaries agents in caries management." Advances in dental research **24**(2): 28-31.

Featherstone, J. D., M. Fontana and M. Wolff (2018). "Novel Anticaries and Remineralization Agents: Future Research Needs." J Dent Res **97**(2): 125-127.

Featherstone, J. D., G. K. Stookey, M. A. Kaminski and R. V. Faller (2011). "Recommendation for a non-animal alternative to rat caries testing." Am J Dent **24**(5): 289-294.

Ferraz, N. K., L. C. Nogueira, M. L. Pinheiro, L. S. Marques, M. L. Ramos-Jorge and J. Ramos-Jorge (2014). "Clinical consequences of untreated dental caries and toothache in preschool children." Pediatr Dent **36**(5): 389-392.

Gao, S. S., S. Zhang, M. L. Mei, E. C. Lo and C. H. Chu (2016). "Caries remineralisation and arresting effect in children by professionally applied fluoride treatment - a systematic review." BMC Oral Health **16**: 12.

Gao, S. S., I. S. Zhao, S. Duffin, D. Duangthip, E. C. M. Lo and C. H. Chu (2018). "Revitalising Silver Nitrate for Caries Management." International journal of environmental research and public health **15**(1): 80.

Gao, S. S., I. S. Zhao, N. Hiraishi, D. Duangthip, M. L. Mei, E. C. M. Lo and C. H. Chu (2016). "Clinical Trials of Silver Diamine Fluoride in Arresting Caries among Children: A Systematic Review." JDR Clin Trans Res **1**(3): 201-210.

Garg, S., A. Sadr and D. Chan (2019). "Potassium Iodide Reversal of Silver Diamine Fluoride Staining: A Case Report." Oper Dent **44**(3): 221-226.

Griffin, S. O., L. Wei, B. F. Gooch, K. Weno and L. Espinoza (2016) "Vital Signs: Dental Sealant Use and Untreated Tooth Decay Among U.S. School-Aged Children." MMWR. Morbidity and mortality weekly report **65**, 1141-1145 DOI: 10.15585/mmwr.mm6541e1.

Gupta, J., M. S. Thomas, M. Radhakrishna, N. Srikant and K. Ginjupalli (2019). "Effect of silver diamine fluoride-potassium iodide and 2% chlorhexidine gluconate cavity cleansers on the bond strength and microleakage of resin-modified glass ionomer cement." J Conserv Dent **22**(2): 201-206.

Hamama, H. H., C. K. Yiu and M. F. Burrow (2015). "Effect of silver diamine fluoride and potassium iodide on residual bacteria in dentinal tubules." Aust Dent J **60**(1): 80-87.

Horst, J. A., H. Ellenikiotis and P. L. Milgrom (2016). "UCSF Protocol for Caries Arrest Using Silver Diamine Fluoride: Rationale, Indications and Consent." Journal of the California Dental Association **44**(1): 16-28.

Horst, J. A., H. Ellenikiotis and P. L. Milgrom (2016). "UCSF Protocol for Caries Arrest Using Silver Diamine Fluoride: Rationale, Indications and Consent." J Calif Dent Assoc **44**(1): 16-28.

Hu, S., B. Meyer and M. Duggal (2018). "A silver renaissance in dentistry." Eur Arch Paediatr Dent **19**(4): 221-227.

Ishiguro, T., G. Mayanagi, M. Azumi, H. Otani, A. Fukushima, K. Sasaki and N. Takahashi (2019). "Sodium fluoride and silver diamine fluoride-coated tooth surfaces inhibit bacterial acid production at the bacteria/tooth interface." J Dent **84**: 30-35.

Johhnson, B. and N. Serban (2019). "Projecting the economic impact of silver diamine fluoride on caries treatment expenditures and outcomes in young U.S. children." **79**(3): 215-221.

Karched, M., D. Ali and H. Ngo (2019). "In vivo antimicrobial activity of silver diammine fluoride on carious lesions in dentin." J Oral Sci **61**(1): 19-24.

Kassebaum, N. J., A. G. C. Smith, E. Bernabe, T. D. Fleming, A. E. Reynolds, T. Vos, C. J. L. Murray, W. Marcenes and G. B. D. O. H. Collaborators (2017). "Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990-2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors." J Dent Res **96**(4): 380-387.

Kielbassa, A. M., S. P. Shohadai and J. Schulte-Mönting (2001). "Effect of saliva substitutes on mineral content of demineralized and sound dental enamel." Support Care Cancer **9**(1): 40-47.

Knight, G. M., J. M. McIntyre, G. G. Craig, Mulyani, P. S. Zilm and N. J. Gully (2005). "An in vitro model to measure the effect of a silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to Streptococcus mutans." Aust Dent J **50**(4): 242-245.

Knight, G. M., J. M. McIntyre, G. G. Craig, Mulyani, P. S. Zilm and N. J. Gully (2009). "Inability to form a biofilm of Streptococcus mutans on silver fluoride- and potassium iodide-treated demineralized dentin." Quintessence Int **40**(2): 155-161.

Knight, G. M., J. M. McIntyre and Mulyani (2006). "The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine." Aust Dent J **51**(1): 42-45.

Levine, M. J. (1993). "Development of artificial salivas." Crit Rev Oral Biol Med **4**(3-4): 279-286.

Li, R., E. C. Lo, B. Y. Liu, M. C. Wong and C. H. Chu (2016). "Randomized clinical trial on arresting dental root caries through silver diammine fluoride applications in community-dwelling elders." J Dent **51**: 15-20.

Li, Y., Y. Liu, W. J. Psoter, O. M. Nguyen, T. G. Bromage, M. A. Walters, B. Hu, S. Rabieh and F. C. Kumararaja (2019). "Assessment of the Silver Penetration and Distribution in Carious Lesions of Deciduous Teeth Treated with Silver Diamine Fluoride." Caries Res **53**(4): 431-440.

Lippert, F. (2017). "Effect of Enamel Caries Lesion Baseline Severity on Fluoride Dose-Response." Int J Dent **2017**: 4321925.

Lippert, F., D. Churchley and R. J. Lynch (2015). "Effect of Lesion Baseline Severity and Mineral Distribution on Remineralization and Progression of Human and Bovine Dentin Caries Lesions." Caries Res **49**(5): 467-476.

Lippert, F., E. E. Newby, R. J. Lynch, V. K. Chauhan and B. R. Schemehorn (2009). "Laboratory assessment of the anticaries potential of a new dentifrice." J Clin Dent **20**(2): 45-49.

Liu, B. Y., E. C. Lo and C. M. Li (2012). "Effect of silver and fluoride ions on enamel demineralization: a quantitative study using micro-computed tomography." Aust Dent J **57**(1): 65-70.

Llodra, J. C., A. Rodriguez, B. Ferrer, V. Menardia, T. Ramos and M. Morato (2005). "Efficacy of silver diamine fluoride for caries reduction in primary teeth and first permanent molars of schoolchildren: 36-month clinical trial." J Dent Res **84**(8): 721-724.

Lo, E. C., C. H. Chu and H. C. Lin (2001). "A community-based caries control program for pre-school children using topical fluorides: 18-month results." J Dent Res **80**(12): 2071-2074.

Lobo, M. M., R. B. Goncalves, G. M. Ambrosano and L. A. Pimenta (2005). "Chemical or microbiological models of secondary caries development around different dental restorative materials." J Biomed Mater Res B Appl Biomater **74**(2): 725-731.

Mei, M. L., Q.-l. Li, C.-H. Chu, E.-M. Lo and L. P. Samaranayake (2013). "Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries." Annals of Clinical Microbiology and Antimicrobials **12**(1): 4.

Mei, M. L., Q.-l. Li, C.-H. Chu, E. C.-M. Lo and L. P. Samaranayake (2013). "Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries." Annals of clinical microbiology and antimicrobials **12**: 4-4.

Mei, M. L., Q. L. Li, C. H. Chu, E. C. Lo and L. P. Samaranayake (2013). "Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries." Ann Clin Microbiol Antimicrob **12**: 4.

Mei, M. L., E. C. M. Lo and C. H. Chu (2018). "Arresting Dentine Caries with Silver Diamine Fluoride: What's Behind It?" J Dent Res **97**(7): 751-758.

Mei, M. L., I. S. Zhao, L. Ito, E. C. Lo and C. H. Chu (2016). "Prevention of secondary caries by silver diamine fluoride." Int Dent J **66**(2): 71-77.

Meyer-Lueckel, H., N. Umland, W. Hopfenmuller and A. M. Kielbassa (2004). "Effect of mucin alone and in combination with various dentifrices on in vitro Remineralization." Caries Res **38**(5): 478-483.

Moynihan, P. and P. E. Petersen (2004). "Diet, nutrition and the prevention of dental diseases." Public Health Nutrition **7**(1A): 201-226.

Nieuw Amerongen, A. V., C. H. Oderkerk and A. A. Driessen (1987). "Role of mucins from human whole saliva in the protection of tooth enamel against demineralization in vitro." Caries Res **21**(4): 297-309.

Oliveira, B. H., A. Rajendra, A. Veitz-Keenan and R. Niederman (2019). "The Effect of Silver Diamine Fluoride in Preventing Caries in the Primary Dentition: A Systematic Review and Meta-Analysis." Caries Res **53**(1): 24-32.

Ramos-Jorge, J., I. A. Pordeus, M. L. Ramos-Jorge, L. S. Marques and S. M. Paiva (2014). "Impact of untreated dental caries on quality of life of preschool children: different stages and activity." **42**(4): 311-322.

Roberts, A., J. Bradley, S. Merkley, T. Pachal, J. Gopal and D. Sharma "Does potassium iodide application following silver diamine fluoride reduce staining of tooth? A systematic review." Aust Dent J **65**(2): 109-117.

Rosenblatt, A., T. C. Stamford and R. Niederman (2009). "Silver diamine fluoride: a caries "silver-fluoride bullet"." J Dent Res **88**(2): 116-125.

Savas, S., E. Kucukyilmaz, E. U. Celik and M. Ates (2015). "Effects of different antibacterial agents on enamel in a biofilm caries model." J Oral Sci **57**(4): 367-372.

Sayed, M., N. Matsui, N. Hiraishi, T. Nikaido, M. F. Burrow and J. Tagami (2018). "Effect of Glutathione Bio-Molecule on Tooth Discoloration Associated with Silver Diammine Fluoride." International journal of molecular sciences **19**(5): 1322.

Scaramucci, T., A. B. Borges, F. Lippert, D. T. Zero, I. V. Aoki and A. T. Hara (2015). "Anti-erosive properties of solutions containing fluoride and different film-forming agents." J Dent **43**(4): 458-465.

Seifo, N., M. Robertson, J. MacLean, K. Blain, S. Grosse, R. Milne, C. Seeballuck and N. Innes (2020). "The use of silver diamine fluoride (SDF) in dental practice." British Dental Journal **228**(2): 75-81.

Selwitz, R. H., A. I. Ismail and N. B. Pitts (2007). "Dental caries." Lancet **369**(9555): 51-59.

Slomiany, B. L., V. L. Murty, J. Piotrowski and A. Slomiany (1996). "Salivary mucins in oral mucosal defense." Gen Pharmacol **27**(5): 761-771.

Sorkhdini, P., Y. O. Crystal, Q. Tang and F. Lippert (2020). "The effect of silver diamine fluoride in preventing in vitro primary coronal caries under pH-cycling conditions." Archives of Oral Biology: 104950.

Sorkhdini, P., R. L. Gregory, Y. O. Crystal, Q. Tang and F. Lippert (2020). "Effectiveness of in vitro primary coronal caries prevention with silver diamine fluoride - Chemical vs biofilm models." Journal of Dentistry **99**: 103418.

Stoodley, P., J. Wefel, A. Gieseke, D. Debeer and C. von Ohle (2008). "Biofilm plaque and hydrodynamic effects on mass transfer, fluoride delivery and caries." J Am Dent Assoc **139**(9): 1182-1190.

Ten Bosch, J. J. and B. Angmar-Mansson (1991). "A review of quantitative methods for studies of mineral content of intra-oral caries lesions." J Dent Res **70**(1): 2-14.

Timms, L., O. Sumner, C. Deery and H. J. Rogers (2020). "Everyone else is using it, so why isn't the UK? Silver diamine fluoride for children and young people." Community Dent Health.

Vasquez, E., G. Zegarra, E. Chirinos, J. L. Castillo, D. R. Taves, G. E. Watson, R. Dills, L. L. Mancl and P. Milgrom (2012). "Short term serum pharmacokinetics of diammine silver fluoride after oral application." BMC Oral Health **12**: 60.

Vieira, A. R., M. Bayram, F. Seymen, R. C. Sencak, F. Lippert and A. Modesto (2017). "In Vitro Acid-Mediated Initial Dental Enamel Loss Is Associated with Genetic Variants Previously Linked to Caries Experience." Front Physiol **8**: 104.

Vinh N, C. N., Joel F, Carolyn P. (2017). "Potassium Iodide. The Solution to Silver Diamine Fluoride Discoloration?" Adv Dent Oral Health **5**(1).

Wang, L. J., R. Tang, T. Bonstein, P. Bush and G. H. Nancollas (2006). "Enamel demineralization in primary and permanent teeth." J Dent Res **85**(4): 359-363.

White, D. J. (1988). "Reactivity of fluoride dentifrices with artificial caries. II. Effects on subsurface lesions: F uptake, F distribution, surface hardening and remineralization." Caries Res **22**(1): 27-36.

Wilson, P. R. and A. D. Beynon (1989). "Mineralization differences between human deciduous and permanent enamel measured by quantitative microradiography." Arch Oral Biol **34**(2): 85-88.

Yu, O. Y., M. L. Mei, I. S. Zhao, Q. L. Li, E. C. Lo and C. H. Chu (2018). "Remineralisation of enamel with silver diamine fluoride and sodium fluoride." Dent Mater **34**(12): e344-e352.

Yu, O. Y., I. S. Zhao, M. L. Mei, E. C. Lo and C. H. Chu (2018). "Effect of Silver Nitrate and Sodium Fluoride with Tri-Calcium Phosphate on Streptococcus mutans and Demineralised Dentine." Int J Mol Sci **19**(5).

Zhang, M., L. B. He, R. A. Exterkate, L. Cheng, J. Y. Li, J. M. Ten Cate, W. Crielaard and D. M. Deng (2015). "Biofilm layers affect the treatment outcomes of NaF and Nano-hydroxyapatite." J Dent Res **94**(4): 602-607.

Zhao, I. S., S. Chu, O. Y. Yu, M. L. Mei, C. H. Chu and E. C. M. Lo (2019). "Effect of silver diamine fluoride and potassium iodide on shear bond strength of glass ionomer cements to caries-affected dentine." Int Dent J **69**(5): 341-347.

Zhao, I. S., S. S. Gao, N. Hiraishi, M. F. Burrow, D. Duangthip, M. L. Mei, E. C. Lo and C. H. Chu (2018). "Mechanisms of silver diamine fluoride on arresting caries: a literature review." Int Dent J **68**(2): 67-76.

Zhao, I. S., M. L. Mei, M. F. Burrow, E. C.-M. Lo and C.-H. Chu (2017). "Effect of Silver Diamine Fluoride and Potassium Iodide Treatment on Secondary Caries Prevention and Tooth Discolouration in Cervical Glass Ionomer Cement Restoration." International journal of molecular sciences **18**(2): 340.

Zhao, I. S., M. L. Mei, M. F. Burrow, E. C. Lo and C. H. Chu (2017). "Effect of Silver Diamine Fluoride and Potassium Iodide Treatment on Secondary Caries Prevention and Tooth Discolouration in Cervical Glass Ionomer Cement Restoration." Int J Mol Sci **18**(2).

Zhao, I. S., M. L. Mei, M. F. Burrow, E. C. Lo and C. H. Chu (2017). "Prevention of secondary caries using silver diamine fluoride treatment and casein phosphopeptide-amorphous calcium phosphate modified glass-ionomer cement." J Dent **57**: 38-44.

Zhao, I. S., M. L. Mei, Q. L. Li, E. C. M. Lo and C. H. Chu (2017). "Arresting simulated dentine caries with adjunctive application of silver nitrate solution and sodium fluoride varnish: an in vitro study." Int Dent J **67**(4): 206-214.

Zhi, Q. H., E. C. Lo and A. C. Kwok (2013). "An in vitro study of silver and fluoride ions on remineralization of demineralized enamel and dentine." Aust Dent J **58**(1): 50-56.

Zhi, Q. H., E. C. Lo and H. C. Lin (2012). "Randomized clinical trial on effectiveness of silver diamine fluoride and glass ionomer in arresting dentine caries in preschool children." J Dent **40**(11): 962-96

CURRICULUM VITAE

Parand Sorkhdini

Education:

2014-2021 Doctor of Philosophy (Ph.D.) in Dental Sciences, Indiana University-Purdue University, School of Dentistry, Indianapolis, Indiana, USA

Dissertation: Primary coronal caries prevention with silver diamine fluoride – Investigations into efficacy and mode of action

2005-2012 Doctor of Veterinary Medicine (DVM), Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

Thesis: The Effect of Radachlorin-Mediated Antimicrobial Photodynamic Therapy on Clinical Parameters and Cytokine Profile in Ligature-Induced Periodontitis in Dogs

Honors and Awards:

1. Fall 2014 to present- Awarded a fellowship from Indiana University School of Dentistry.
2. Fall 2019- Travel award from "The University of Tennessee Health Science Center College of Dentistry - Hinman Student Research Symposium", Memphis, TN.
3. Spring 2020- Scholarship award from the Graduate and Professional Educational Grant (GPEG) committee for attending 2020 the International Association for Dental Research (IADR).
4. Spring 2019- Scholarship award from the GPEG committee for attending "The 107th Thomas P. Hinman Dental Meeting, March 2019.

5. Fall 2016 to Spring 2020- Received a scholarship from “Graduate Professional Student Government” (GPSG) for being an active representative for IUSD graduate student (For 8 Semesters).

6. September 2010- Awarded a grant from” The Laser Research Center of Dentistry Tehran University of Medical Sciences, for DVM dissertation project in a collaboration with School of Dentistry, Tehran University of Medical Sciences, number: 88-04-97-9743.

Publications:

1. Sorkhdini P, Gregory RL, Crystal YO, Tang Q, Lippert F. Effectiveness of in vitro primary coronal caries prevention with silver diamine fluoride - Chemical vs biofilm models. Journal of dentistry. 2020;99:103418.

2. Sorkhdini P, Crystal YO, Tang Q, Lippert F. The effect of Silver Diamine Fluoride in Preventing In Vitro Primary Coronal Caries under pH-cycling Conditions, Archives of Oral Biology, 2020:104950.

3. Sorkhdini P, Crystal YO, Tang Q, Lippert F. Rehardening Effect of Silver Diamine Fluoride with and without Mucin on Early Enamel Caries Lesions. International Dental Journal. January 2020, Submitted.

4. Sorkhdini P, Crystal YO, Tang Q, Lippert F. The Effect of Silver Diamine Fluoride on the Remineralization of Early Enamel Caries Lesions under pH-cycling Conditions. Australian Dental Journal, to be submitted.

5. Utreja A, Bain C, Turek B, Holland R, AlRasheed R, Sorkhdini P, et al. Maxillary expansion in an animal model with light, continuous force. Angle Orthod. 2018;88(3):306-13.

6. Sorkhdini P, Moslemi N, Jamshidi S, Jamali R, Amirzargar AA, Fekrazad R. Effect of hydrosoluble chlorine-mediated antimicrobial photodynamic therapy on clinical parameters and cytokine profile in ligature-induced periodontitis in dogs. J Periodontol. 2013;84(6):793-800.

Oral Presentations:

1. Sorkhdini P., Gregory RL., Lippert F. In Vitro Primary Coronal Caries Prevention with Silver Diamine Fluoride. 2019 International Association for Dental Research (IADR/AADR/CADR), Vancouver Convention Centre, Vancouver, BC, Canada, June 2019.

2. Sorkhdini P. The effectiveness of SDF in preventing enamel demineralization with chemical models. Indiana University School of Dentistry Student Research Presentation Program (SRPP), October 2018.

3. Sorkhdini P. Isolation and Quantification of periodontal pathogens by using 16s rRNA from salivary epithelial cells. Indiana University School of Dentistry SRPP, February 2017.

4. Sorkhdini P. Maxillary Expansion in a Rat Model: Histological and Micro-CT analysis of bone. Indiana University School of Dentistry SRPP, June 2016.

5. Sorkhdini P. Binding of Porphyromonas gingivalis to Streptococcus gordonii cells grown in the presence of different concentrations of nicotine. Indiana University School of Dentistry SRPP, July 2015.

6. Sorkhdini P. Fekrazad R., Jamshidi SH., Moslemi N., SalariSedigh H. A novel technique to induce experimental periodontitis in dogs. The 3rd International Symposium

of Veterinary Surgery & The 9th Iranian Symposium of Veterinary Surgery, Anesthesia and Radiology, Kish Island, Persian Gulf, Iran, April 2011.

7. Sorkhdini P., Molazem M., Ramezani N., Vajhi A. Three- dimensional color and power

Doppler Ultrasonographic finding in hemangiosarcoma spleen tumor and compare it with benign spleen masses in dog", The 16th Iranian Veterinary Congress, Tehran, Iran, April 2010.

8. Sorkhdini P., Sasani F. Clinical and histopathological evaluation of fowl pox virus. Student Scientific Presentations, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran, Spring 2008.

Poster Presentations:

1. Sorkhdini P., Gregory RL., Tang Q., Lippert F. Caries Prevention with Silver Diamine Fluoride Studied Under pH-cycling Conditions. The 28th Indiana University School of Dentistry Research Day, April 2020 (Digital presentation)

2. Sorkhdini, P., Gregory RL., Martinez-Mier, EA., Crystal. OY., Stelzner S., Tang Q., Lippert F. Caries Prevention with Silver Diamine Fluoride Studied Under pH-cycling Conditions. 2020 the International Association for Dental Research (IADR), Washington, D.C. March 2020 (Digital presentation)

3. Sorkhdini P., Gregory RL., Lippert F. Comparison Between Biofilm and Chemical Models in In Vitro Primary Coronal Caries Prevention with Silver Diamine Fluoride, The 25th Hinman Student Research Symposium", Memphis, TN. November 2019.

4. Sorkhdini P., Gregory RL., Lippert F. In Vitro Primary Coronal Caries Prevention with Silver Diamine Fluoride. The 27th Indiana University School of Dentistry Research Day, April 2019.

5. Sorkhdini P., Gregory RL., Lippert F. In Vitro Primary Coronal Caries Prevention with Silver Diamine Fluoride. The 2019 Indiana Branch of American Society for Microbiology (IBASM) Annual meeting, Brown County, IN. April 2019.

6. Sorkhdini P., Moslemi N., Jamshidi SH., Amirzargar A., Ferkrazad R. A New Model to Induce Chronic Experimental Periodontitis in Dogs. The 26th Indiana University School of Dentistry Research Day, April 2018.

7. Sorkhdini P., Staller S., Lindsay A., Srinivasan M. Determination of Periodontal Pathogens by Using 16srRNA from Salivary Epithelial Cells. Indiana University–Purdue University Research Day, April 2017.

8. Sorkhdini P., Staller S., Lindsay A., Srinivasan M. Determination of Periodontal Pathogens by Using 16srRNA from Salivary Epithelial Cells. The 25th Indiana University School of Dentistry Research Day, April 2017.

9. Sorkhdini P., Bain C., Utreja A. Maxillary Expansion in the Rat Model: Histological and Micro-CT Analysis of Bone. The 25th Indiana University School of Dentistry Research Day, April 2017.

10. Sorkhdini P., Grace Gomez F G., Gregory R L. Binding of *Porphyromonas gingivalis* to *Streptococcus gordonii* Cells Grown in the Presence of Nicotine. The 24th Indiana University School of Dentistry Research Day, April 2016.